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DEVELOPMENT OF DUMONTIA FILIFORMIS[1]

II. DEVELOPMENT OF SEXUAL PLANTS AND GENERAL DISCUSSION OF RESULTS[2]

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(WITH PLATES XIX–XXII AND SEVEN FIGURES)

Introduction

Dumontia filiformis (Huds.) Grev. is a red seaweed which is widely scattered in the temperate zones. It has been reported as occurring on the Auckland and Falkland Islands (**2**), on the shores of Alaska, and is very common in northern Europe. This species was first found on the Atlantic coast of North America, by the writer, at South Harpswell, Maine, in June 1913. Tetrasporic and cystocarpic plants were collected at that time. Sterile plants were collected by THAXTER at Kittery Point, Maine, in April 1914.[3] These are the only two points on this coast where plants of *Dumontia* have been reported to occur.

In all probability *Dumontia* has become established on the coast at South Harpswell some time between 1909–1913. F. S. COLLINS collected at South Harpswell in the early part of July for 6 years (1902–1905 and 1908–1909) in the same pools in which *Dumontia* was abundant in July 1913 and 1914. He states that he has never found a single specimen of *Dumontia* in any of these pools, and if

[1] Botanical contribution from the Johns Hopkins University, no. 55.

[2] First paper entitled "The development of the tetraspores." Plant World, **19**: 271–281. *figs.* 2. 1916.

[3] Personal letter from F. S. COLLINS.

the plants were then present they must have been extremely scarce. The plants were very abundant in the early part of July 1913. If a few solitary plants were present in 1909, it is apparent that they must have multiplied rapidly in the following 4 years. It is highly improbable, therefore, that any plants of *Dumontia* were present at South Harpswell as early as 1905.

GREVILLE (1) in 1830 described fructifications which he had observed in *Dumontia filiformis*. These fructifications were attached to the inner surface of the wall of the thallus and consisted of "clusters of large ovate seeds." It is evident from GREVILLE'S description and figures that these "seeds" were carpospores. KÜTZING (5) published illustrations and a very brief description of the tetraspores. HARVEY (2) pictures a group of carpospores and states that "clustered spores are common." THURET (17) refers to the antheridia of *Dumontia*, so at that time these bodies were known to exist. The writer has not been able to find any description of the antheridia. All the papers published on the red algae previous to 1883 dealt chiefly with the distribution and seasonal occurrence of the various genera and the gross morphology of the individuals. SCHMITZ'S (13) paper in 1883 marks a greater step in advance in the study of the red algae than has since been made by any one investigator. Although his descriptions are not complete, his general conception of the structure of the female reproductive organs of *Dudresnaya*, *Glocosiphonia*, and other members of the Cryptonemiales is essentially correct in regard to the cell history. His observations on *Dudresnaya*, *Polyides*, and *Petrocelis* concerning the behavior of the nuclei in the ooblastema filaments and auxiliary cells are correct. In *Glocosiphonia* and some other genera SCHMITZ reports that the nucleus in the cell which forms the carpospores is the product of two fusions.

The structure of the female reproductive organs of the red algae is quite complicated. The auxiliary cell, the cell which produces the carpospores, in nearly all the genera is formed by the fusion of the cytoplasm of two or more cells. The behavior of the nuclei in these cells fusing to form the auxiliary cell proved to be a stumbling block to SCHMITZ and many other workers, some of whom regarded the nucleus in this cell as the product of as many as 6 fusions (HAUPT-

FLEISCH 4). The next epoch making paper in the study of the red algae was that by OLTMANNS (9). OLTMANNS worked out very carefully and in much detail the nuclear and cell history during fertilization and carpospore formation in *Dudresnaya*, *Gloeosiphonia*, and *Dasya*. OLTMANNS' chief contribution was the convincing evidence that the nucleus functioning in the auxiliary cell at the time of the formation of the carpospores is a descendant of the fusion nucleus in the carpogonium, and that no other nuclear fusion has occurred. OLTMANNS' descriptions are detailed and his illustrations are remarkably clear, but nevertheless some present day botanists question his observations concerning the absence of a fusion between the nucleus in the auxiliary cell and that nucleus which enters it from the sporogenous filament. These botanists are inclined to believe that in the members of the Crytonemiales, as in certain of the Ascomycetes, there are two nuclear fusions at the time of fertilization. *Dumontia* and *Dudresnaya* belong to the same family, Dumontiaceae, and it is to be expected therefore that the two genera will have similar reproductive organs. In view of the fact that OLTMANNS' results have been questioned by some workers, the present investigation of *Dumontia filiformis* was undertaken for the purpose of gaining all possible information concerning the behavior of the nuclei during fertilization and the formation of the carpospores. It was also desired to gain information concerning the general structure of this alga, the cytology of its tetraspores, and the structure of its male reproductive organs.

This study was begun in June 1913, at the Harpswell Laboratory, South Harpswell, Maine, where the plants were abundant. It was continued during 1913, 1914, and 1915 at South Harpswell and at Johns Hopkins University.

The writer wishes to thank Professor J. S. KINGSLEY for the privileges of the Harpswell Laboratory, and also Dr. M. A. HOWE and Mr. F. S. COLLINS for identifying this alga. This investigation was undertaken at the suggestion of Professor D. S. JOHNSON, under whose directions it has been carried out, and whose criticisms have been a constant source of aid. Dr. W. D. HOYT also has kindly examined many of the preparations.

Methods

Plants of this alga, either whole or cut into lengths of 5–10 mm. each, were fixed in medium chromo-acetic solution, or in Flemming's fluid, within a few minutes after being collected. As the alga is very gelatinous, great care was taken that all changes in the alcohols should be made very gradually. The material on which the alcohol was changed in 5 per cent grades showed considerably less shrinkage than that on which the changes were made in 10 per cent grades. Most of the paraffin sections used were 10 or 12 μ thick. Sections 2 μ thick were also used for cytological details. For staining, Heidenhain's iron alum hematoxylin (1 hour in alum solution, 2 hours in hematoxylin) gave the best results. Acid fuchsin and methyl green stained the spores very well, but were not satisfactory for the vegetative structure. The triple stain, safranin, gentian-violet, and orange G, was also used. The slipping from the slide of sections of material fixed in Flemming's fluid occurred somewhat frequently in consequence of bleaching the sections in hydrogen peroxide. This difficulty was finally largely overcome by dipping the slides into 0.5 per cent solution of celloidin in a mixture of equal parts of alcohol and ether.

Description

HABITAT AND APPEARANCE

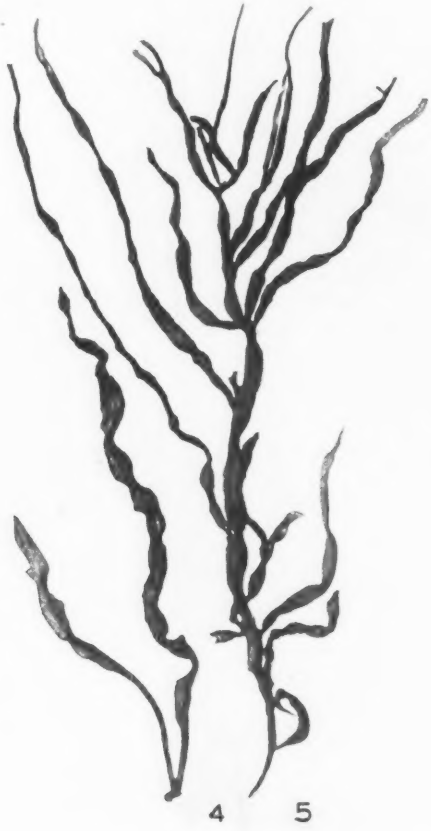
Dumontia, at South Harpswell, grows in abundance in tufts in the small tide pools and also on the rocks that are exposed to the air at low water. On large round rocks which were much exposed to the surf, female and tetrasporic plants of *Dumontia* were found growing down almost to the lower limit reached by *Chondrus crispus*, that is, just below the mean low water level. There is considerable variation in the size of the plants. The larger plants were found in the more exposed places. The plants in the tide pools near the low water mark were larger than the plants in the higher pools, and the largest plants of all were those growing at low levels on the round rocks. The color of the plants varies from a rich dark red to a pale reddish yellow. Mature tetrasporic and female plants ranged in height from 4 cm. to 23 cm. There is

apparently no regular or constant system of branching, and the number of branches present is not related to the height of the plant (figs. 1–7). The plants shown in figs. 1 and 2 have almost

FIGS. 1–3.—Mature female plants showing cystocarps imbedded in thallus

the same number of branches, and their respective heights are 7 cm. and 19 cm. All the cystocarpic plants found were branched. Tetrasporic plants were found which were 12 cm. in height and

were unbranched. The female plants evidently attain practically their maximum size before the carpogonial branches are initiated. The average size of the female plants collected on April 12 was the



FIGS. 4, 5.—Mature tetrasporic plants branched and unbranched showing fraying out of thallus at apices of branches and main axis; ×0.6.

same as that of the mature cystocarpic plants collected in June. Some of these plants collected in April bore only young carpogonial branches, while others bore mature branches of this type and

auxiliary cell apparatuses in the upper portion of their thalli. Carpogonial branches therefore were probably initiated on these plants only a few days before they were collected. The average

FIG. 6.—Tetrasporic plant showing much inflated main axis and branches; ×0.5

size of the male is less than that of the female plants. The maximum height of the male plants examined was 20 cm. They could be distinguished from the young female plants only by microscopical

examination. Female plants bearing mature cystocarps can readily be distinguished from the male and tetrasporic plants because the cystocarps form protrusions in the wall of the thallus (figs. 1–3).

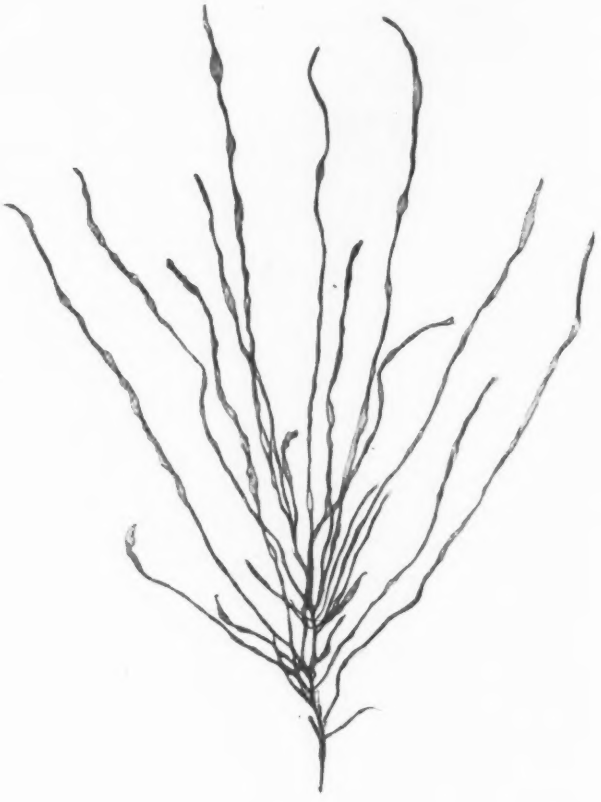


FIG. 7.—Tetrasporic plant showing large number of much twisted branches; ×0.4

The ends of the majority of plants collected in June and July were considerably frayed out (figs. 1, 3, 4, 6). Since growth is apical, the branches cannot increase greatly in length after the fraying has begun.

LEWIS (8) has shown that the sexual and asexual generations in most of the Florideae at Woods Hole differ physiologically, but are identical in vegetative structure, chromosome number of course excepted. These two generations in many forms appear at different seasons and have a tendency to grow on different kinds of substrata. The plants shown in figs. 2 and 5 are fair examples of well developed cystocarpic and tetrasporic plants of *Dumontia filiformis*. It is evident from a comparison of these plants that in this species also the two generations are morphologically almost identical. The tetrasporic and cystocarpic plants of *Dumontia*, so far as substrata on which they grow are concerned, appear to be physiologically identical also. The two kinds of plants grow together on all the large rocks and in most of the tide pools. The tetrasporic plants were more abundant on the whole than the cystocarpic ones, and a few pools contained only the former. These tide pools, however, were in every instance within 3 or 4 feet of apparently similar pools on the same level, in which both kinds of plants grew. The temperature of the water was taken in a number of the pools. It was found that the temperature of pools in the same vicinity did not vary more than 2° C. This difference existed between pools which contained tetrasporic plants only, as well as between these and those pools in which both kinds of plants were present.

SEASONAL OCCURRENCE

Male and young female plants of *Dumontia* were collected in the latter part of April 1914 and on April 12, 1915. An unsuccessful search was made for plants in January 1914. It is believed that the plants were not then present. The ratio of female to male plants in the collection made in April 1914 could not be determined, owing to the fact that the plants when examined were considerably broken up. The ratio of female to male plants in the collection of April 12, 1915, was 3 to 1. This ratio is based upon the examination of 24 plants collected from several different tide pools. Another collection of plants was made on April 26, 1915, and of these plants 24 were examined, all of which proved to be female. Of these 24 plants 10 bore only carpogonial and auxiliary cell branches, while 14 bore chiefly young cystocarps and auxiliary cell branches.

There were a few plants in the collection made on April 12 which bore no reproductive organs; these were probably female plants in which the carpogonial branches had not yet been initiated. All of the plants over 4 cm. in height, collected in June and July, with the exception of 2 or 3 individuals bearing carpogonial branches, bore either mature cystocarps or tetraspores. Hundreds of plants were collected and a careful but entirely unsuccessful search was made for individuals bearing spermatia. It seems evident from these facts that the male plants are present for only 2 or 3 weeks in April. It is possible, of course, that a few solitary individuals were present in June and July. This view is supported by the fact that it was possible to find on the female plants collected during this time all stages from the 1-celled carpogonial branch to the mature cystocarps. The cystocarpic plants reach their maximum development in the early part of June and have completely disappeared by the middle of July. The tetrasporic plants attain their maximum development in the latter part of June, although plants 19 cm. in height were very abundant as early as June 12. A few tetrasporic plants persist until late in August, but they are rare even in the latter part of July. The tetrasporic and female plants in all the red algae seem to be more numerous than the male plants. The experiments of LEWIS (**7**) with *Griffithsia Bornetiana* and *Dasya elegans* indicate that in both of these species the tetraspores form equal numbers of male and female plants. This is probably true of *Dumontia* and other members of the Florideae. The apparent scarcity of male plants may be due to the fact that in some forms they are exceedingly small and therefore are easily overlooked. This would not apply to such forms as *Dumontia*, however, in which the difference in the average height of the male and female plants is not more than 4–5 cm. SVEDELIUS (**14, 16**) reports that the male plants of *Martensia* and *Delesseria* die shortly after they have discharged their spermatia. This is probably true of the *Dumontia* plants also. LEWIS (**8**) has found that the tetrasporic plants of most of the red algae at Woods Hole are very abundant in July. The tetraspores germinate to form cystocarpic plants from which carpospores are released in September. The holdfasts of young sporelings formed from these

carpospores persist through the winter. These holdfasts, in the following June, produce adventitious shoots which develop into tetrasporic plants. LEWIS believes that this is in general the seasonal cycle of many of the Florideae, but states that "there are also exceptions to the separation in point of time of the two generations. This separation is never of a perfectly sharp and definite character, as the generations always overlap to a certain extent in midsummer."

The seasonal cycle of *Dumontia* at South Harpswell is evidently not similar to that of the algae just mentioned. The carpospores discharged in May and June apparently develop immediately into the tetrasporic plants which are present in June and July. This seems clear from the fact that the carpospores sometimes germinate even before escaping from the cystocarp, also that young tetrasporic plants 3–7 cm. in height were often found growing beside the stumps of the frayed off cystocarpic plants. Germination of the tetraspores has not been seen, but the occurrence of only male and female plants in the spring would indicate that this species must persist through the winter in the form of sporelings derived from the tetraspores discharged in June and July.

VEGETATIVE STRUCTURE

The holdfast of *Dumontia* is a platelike body composed of a single layer of horizontal filaments, each cell of which produces an ascending, vertical branch (figs. 8, 9). These vertical branches are closely packed together, averaging about 12 cells in length, and are very regular in form and arrangement. They are generally dichotomously branched. The cells of the horizontal filaments usually form no descending branches. The few branches of this character observed consisted of only one cell (fig. 8). It is evident from the size and arrangement of the cells in the ascending branches that they develop by apical growth.

A group of vertical branches in the holdfast elongates to form the upright portion of this alga (fig. 9). In such longitudinal filaments there is a gradual increase in the length of the cells. They are closely packed together at the base of the main axis, forming a solid tissue (fig. 9). At about 0.1 mm. above the holdfast these

longitudinal filaments or medullary hyphae separate, forming a cavity which extends nearly to the tip of the plant. The thallus of the plant is thus tubular in structure. The wall consists of 3 tissue layers. The inner layer is composed of 3 or 4 vertical rows of medullary hyphae. Each cell of a medullary hypha produces a radial branch. These radial rows of cells by repeated dichotomous branching, in planes parallel and perpendicular to the surface of the thallus, form the subcortex and the cortex (fig. 10). A branch arising from a cell of a medullary hypha terminates in 64–128 cortical cells. The cells in the inner subcortex are not closely packed together. The number of cells in a given area increases as a result of the repeated branching, and thus a compact cortex is formed. The 4 figures for each cell type in the following table indicate the two diameters of the cell as seen in a longitudinal section of the thallus, also the diameters of the nucleus and nucleolus.

Medullary hyphae	52.2 μ	8.7 μ	2.7 μ	1.0 μ
Larger subcortical cells	31.5 "	21.5 "	2.7 "	1.0 "
Smaller subcortical cells	11.2 "	9.1 "	1.8 "	0.8 "
Cortical cells	8.4 "	7.0 "	2.8 "	1.4 "

In addition to the radial branches forming the subcortex and cortex, the medullary hyphae may give rise to other branches which remain axial and thus form longitudinal filaments. The medullary hyphae at the tips of the branches and the main axis terminate in a number of short branches composed of small cells (fig. 11). No single initial cell could be recognized at the apex of any branch in *Dumontia*. Branches varying in length from 2 mm. to 4 cm. were examined. The structure of the apex of a branch of *Dumontia* appears to be similar to that of *Furcellaria* (WILLE **18**). *Furcellaria* is cited by both WILLE and OLTMANNS as a good illustration of the "Spring-brunnen" type of vegetative structure (OLTMANNS **9**). The holdfast of *Dumontia* also resembles that of *Furcellaria*. Each of the medullary hyphae in *Dumontia*, as well as each of the lateral branches arising from these hyphae, has its own initial cell. Practically all the vegetative cells of *Dumontia* are uninucleate. All the chromatin in the resting nucleus is in the nucleolus. All the vegetative cells in the thallus, with the possible exception of a

few cells in the lower layers of the holdfast, contain but one chromatophore. The chromatophore is a clathrate hollow ellipsoid lying just inside the cell wall (fig. 12). It is similar to the peripheral portion of the chromatophore figured by WOLFE (**19**) in *Nemalion*. The chromatophore in some cells was seen to be enveloped by a thin layer of cytoplasm in the form of a coarse net. This cytoplasmic envelope, although not always visible, was undoubtedly present in all the cells.

Intercellular connections, such as are characteristic of the Florideae, are present between all the vegetative cells and all the sexual reproductive cells, including the carpospores, until they are almost mature. At each intercellular connection there are two similar disks joined by an apparently homogeneous strand of cytoplasm. The cytoplasm appears to penetrate these disks, but the matter has not been thoroughly investigated. The disks stain readily with hematoxylin, and in some cells appear to be composed of granules (fig. 13). One case was seen in which a strand of cytoplasm 4 μ wide connects two carpospores 22 μ in diameter (fig. 14). In this strand of cytoplasm are several granules having an average length of 0.7 μ. These granules stain with the same intensity as the disks. It is probable that these granules would collect together to form the two disks when the strand of cytoplasm has assumed its normal size. Trichomes are found on all parts of the surface of the thallus. They seem to be most numerous at the base of the frond. They are present on the male, female, and tetrasporic plants. The trichomes are very abundant on the young plants collected in April.

SPERMATIA

Definition.—If the following discussion is to be intelligible, it will be necessary to define the terms which will be used in the description of the male reproductive organs. Those cells which are analogous to the sperms of the green and brown algae will be designated as spermatia. YAMANOUCHI (**20**), writing of *Polysiphonia*, calls these cells sperms, but "spermatia" is the term which has been most widely used by workers on the red algae and is therefore to be preferred.

SCHMITZ (13), WOLFE (19), and some other workers on the red algae have found that the spermatium is sometimes discharged as a naked protoplast. SVEDELIUS (14) therefore maintains that a distinction should be made between the free spermatium, the naked protoplast, and this same protoplast inclosed in a cell wall as it is when attached to the parent plant. He refers to the protoplast inclosed in the cell wall as the "spermatangium." The cell which SVEDELIUS refers to as the "spermatangium mother cell" is analogous to the "spermatium mother cell" of *Nemalion* (WOLFE 19).

Spermatium mother cells.—The spermatium mother cells of *Dumontia filiformis* are homologous to the outer cortical cells of the tetrasporic and cystocarpic plants. In a mature male plant of *Dumontia* almost all the outer layer of cells of all the branches and of the entire main axis from about 1 cm. above the holdfast consists of spermatia and their mother cells. The outer cortical cells of the main axis just above the holdfast are similar to those of the tetrasporic and cystocarpic plants. Although the distribution and position of the spermatium mother cells on the individuals of the different genera varies considerably, no other form has been reported in which they form a continuous layer over almost the entire thallus as they do in *Dumontia*. Each stalk cell in *Dumontia* bears at least two and probably more spermatium mother cells (fig. 15). The spermatium mother cell may bear two spermatia, just as it does in *Polysiphonia* (YAMANOUCHI 20), *Martensia* (SVEDELIUS 14), and *Delesseria* (SVEDELIUS 16).

A distinct chromatophore is certainly present in the stalk cell of the spermatium mother cell of *Dumontia filiformis* (fig. 15). A chromatophore is occasionally seen at the base of a spermatium mother cell borne on one of these stalk cells. The upper part of such a mother cell contains only granular cytoplasm (fig. 19). Many of the mother cells contain only cytoplasm and no chromatophores (fig. 15, first cell to right). Although it was not visible, a net of cytoplasm is undoubtedly present in the stalk cells as it is in all the vegetative cells of *Dumontia*. When the spermatium mother cell was first formed, it must have contained a chromatophore which had been cut off from that in the stalk cell. A large portion of the granular cytoplasm in the spermatium mother cell

was probably present originally in the chromatophore. The presence or absence of chromatophores in these cells could have been more readily determined if living plants had been available for examination. However, even in the preserved material it should be possible to distinguish the chromatophores from the cytoplasm. The protoplasm of the chromatophores is apparently homogeneous; they contain no visible vacuoles and have a definite outline. Many spermatium mother cells were seen which showed intermediate stages in the disappearance of the chromatophore and the formation of the granular cytoplasm (fig. 19).

OSTERHOUT (**11**) states that a reduced chromatophore is present in the young spermatium of *Batrachospermum*. This chromatophore disappears when the young spermatium matures. WOLFE (**19**) observed the division of the chromatophore in the spermatium mother cell of *Nemalion* in preparation for the formation of the spermatium. The chromatophore is for a time visible in the young spermatium and then disappears. Immediately after its disappearance a mass of deep staining cytoplasm is seen at one end of the spermatium. WOLFE believes that at least a portion of this cytoplasm has been derived from the protoplasm of the chromatophore. No other workers, with the possible exception of YAMANOUCHI, have seen chromatophores in the spermatia or in their mother cells. YAMANOUCHI (**20**) states that the sperm mother cells contain fine granular cytoplasm and generally no plastids. Chromatophores are present in all the genera, either in the immediate or somewhat remote ancestors of the spermatium mother cells. SVEDELIUS (**14**) states that he did not actually observe the disappearance of chromatophores in *Martensia*, but he believes that the protoplasm in the chromatophores of certain cells is used in forming the granular cytoplasm of their daughters which do not contain any chromatophores.

Spermatia.—No stages were seen showing a uninucleate spermatium mother cell. This cell in the earliest stages observed is binucleate (fig. 16). The first spermatium is cut off obliquely (figs. 15, 17, 19). The mother cell than elongates, again becomes binucleate (fig. 17), and a second spermatium is cut off. Many spermatia were seen in the swollen gelatinous sheath enveloping

the thallus and some which had actually reached the exterior (fig. 18). Every spermatium seen outside the parent plant is inclosed in a cell wall (fig. 41). No empty cell walls were seen attached to the spermatium mother cell. Many spermatia were seen lying close to the mother cells, but not attached to them (fig. 19). The spermatia in *Dumontia* are apparently cut off from the mother cell in the same manner as they are in *Polysiphonia* (YAMANOUCHI **20**). The wall of the spermatium in both of these genera is a portion of the wall of the spermatium mother cell, and no body is formed which would be homologous to the spermatangium of *Delesseria* (SVEDELIUS **16**). SVEDELIUS (**14**) believes that the spermatia in *Martensia* are set free in the same way as they are in *Polysiphonia*. LEWIS (**6**) states that in *Griffithsia* the spermatia are cut off from the mother cells. This form can hardly be compared with those previously mentioned, because in *Griffithsia* none of the cells of the antheridial filament form cellulose walls, but all are imbedded in the swollen wall of the mother cell of the branch.

The spermatia of *Dumontia*, as far as their contents are concerned, are similar to most of those which have been described in the other genera. The cytoplasm is much vacuolated at the proximal end of the spermatium and is very dense at the distal end. It is difficult to determine the structure of the nucleus, because it is situated at the distal end of the spermatium, imbedded in the dense, deep-staining cytoplasm. All the chromatin appears to be in the nucleolus or in several chromatin granules collected in the center of the nucleus (figs. 17, 19).

CARPOGONIAL BRANCHES

Nearly all the carpogonial branches found in the mature female plants were between the levels of 7.5 and 17.5 mm. from the holdfast. At levels higher up in the thallus, where mature cystocarps occur, a few carpogonial branches are occasionally present. These are not confined to one side of the thallus, but are scattered indiscriminately among the cystocarps. Young cystocarpic plants about 3 cm. in height were occasionally found even as late as July 5. The few cystocarps which were present on these plants were at the tips of the branches. Carpogonial branches were found in the

lower portions of the branches and in the main axis. The carpogonial branches in *Dumontia* evidently are not formed in acropetal succession. The carpogonial branches arise from the lateral branches of the medullary hyphae. They arise either from the basal cells of these subcortical branches or from cells intermediate in position between the medullary hyphae and the surface of the thallus. On the young plants every second or third large subcortical cell or occasionally each successive cell produces a carpogonial branch. Radial branches arise from the intervening cells. In mature plants, where carpogonial branches occur only at the base of the thallus, they develop from every fourth, fifth, or sixth cell. Sometimes the same cell will produce two carpogonial branches or one carpogonial branch and one radial branch (fig. 20).

A mature carpogonial branch consists of 6 or 7 cells and a trichogyne. If there are only 6 cells, they are all in a row. When the carpogonial branch is composed of 7 cells, one cell may be formed as a lateral outgrowth of the basal cell. For convenience and clearness the cells of the carpogonial branch will be numbered. The basal cell which is attached to the vegetative cell will be numbered 1, the cell above it 2, etc. The first cell of the carpogonial branches arises as a conical protrusion of the subcortical cell (fig. 21). A portion of the peripheral chromatophore of the latter is cut off in this protrusion. This first cell is uninucleate (fig. 22), and divides by a wall parallel to its base (fig. 23). The chromatophore in each of these young cells of the carpogonial branches is always peripheral, as it is in all the vegetative cells. The second cell next divides transversely, thus forming a 3-celled carpogonial branch (fig. 24). No data were obtained concerning the details of nuclear division in these earlier stages. This is due to the fact that these stages persist for only a short time, and that the cells are small and almost completely lined by the chromatophores. Considering the size and position of the cells in these young carpogonial branches, it is evident that it must be the terminal cell which divides each time. The cell wall separating the second and third cells was barely visible in some carpogonial branches which had just reached the 3-celled stage (fig. 24). These cells are separated considerably at a slightly later stage (fig. 25). In the 4-celled stage

also the 2 terminal cells are at first in close contact, but later become separated (fig. 26).

The cells of the carpogonial branch in the 4-celled stage may lie in a straight line, or the axis of the 3 terminal cells may form more or less of a right angle with that of the basal cell (figs. 27, 28). The nuclei here furnish evidence to support the assumption that the carpogonial branch develops by the repeated division of the terminal cell. In several cases the nucleus of this cell is considerably enlarged and is evidently just preparing for division (fig. 28). The chromatophores are much more openly clathrate in the 5-celled carpogonial branch than in the younger branches (fig. 29). One branch was observed in which the fifth cell, the terminal cell, was binucleate (fig. 30). Each cell in a carpogonial branch until it has reached the 5- or 6-celled stage generally contains one chromatophore. The one chromatophore then divides into a number of small parts which are connected by strands of cytoplasm (fig. 31). The structure and the arrangement of the cytoplasm and chromatophores at this stage appear to be very similar to those in the tetrasporangium and the tetraspores. The fate of most of the chromatophores in the cells of the carpogonial branch appears to be the same as that of those in the spermatium mother cells. The chromatophores disappear and at the same time the granular cytoplasmic contents of the cells increase. The protoplasm in the chromatophores is apparently used to form a part of the granular cytoplasm. There are generally present 2 or 3 chromatophores in each of the 3 or 4 basal cells, even after fertilization, when the sporogenous filaments are being formed (fig. 42). These chromatophores are hollow ellipsoids, like those in the tetraspores, but unlike the latter generally show no sign of being clathrate.

A large number of carpogonial branches were observed which bore short stumps or fairly long pieces of trichogynes (figs. 32–38). These trichogynes could often be traced almost to the surface of the thallus (figs. 35, 37). Other trichogynes were found which projected beyond the surface of the thallus and which could be traced back toward carpogonial branches (figs. 39, 40). Although no carpogonial branch was found in which the trichogyne could be traced from the carpogonium out beyond the surface of the thallus,

it is evident that this is actually its course. The failure to obtain a satisfactory section was due to the varying and indirect course of the trichogyne. Though most of the sections examined were 12 μ thick, the trichogyne nearly always passed out of the section and it was very difficult to locate it in the adjoining sections. The trichogyne, just beyond its point of attachment to the carpogonium, is often much coiled (figs. 34, 37, 40). The trichogyne is always surrounded by a fairly thick gelatinous wall which is a continuation of that of the carpogonium (figs. 34, 37). The granular cytoplasmic content of the trichogyne stains with the same intensity as does that of the terminal cells of the carpogonial branch. No structure was seen in any trichogyne which could positively be identified as a nucleus. In a few cases a body was seen which appeared to be similar to a nucleolus (fig. 39). This body is surrounded by a light area, but not by a definite membrane, and is therefore not thought to be a nucleus. There are present in some of the trichogynes (fig. 37) 2 or 3 masses which, with hematoxylin, stain like chromatin. The question of the presence of a nucleus in the trichogyne of the Florideae is still unsettled. SVEDELIUS reports that the trichogyne nucleus in *Delesseria sanguinea* disintegrates before fertilization and the chromatin granules pass out into the cytoplasm. It is possible that some of the granules seen in *Dumontia* and other forms are chromatin granules of similar origin.

There are two types of mature carpogonial branches. A cell is sometimes formed as a lateral outgrowth of the basal cell of the carpogonial branch (fig. 35). The cell thus formed is a supernumerary cell and will not be numbered, as it is not always present and has no special function. This supernumerary cell has never been observed in a carpogonial branch which is not mature. The basal cell of the carpogonial branch is often found to be binucleate (figs. 35, 38, 40) and sometimes contains as many as 3 nuclei (fig. 36). The basal cell is sometimes binucleate after having cut off the supernumerary cell (fig. 35). It thus appears that there is a tendency of the basal cell to form a lateral branch. Also the third cell of some of the carpogonial branches appears to be binucleate (figs. 33, 38). It is difficult to determine whether the nucleus in

these cells has actually divided or has merely elongated. The nuclei of cells 1, 2, and 3 of the carpogonial branch are often not in the resting condition, that is, all the chromatin is not in the nucleolus. The chromatin in these nuclei may be in one body surrounded by a number of small granules (fig. 32, cells 1, 2; fig. 38, cell 1), or in several small bodies (fig. 32, cell 3; fig. 36, cell 3; fig. 37, cell 3). The 3 terminal cells (4–6) are smaller than the first 3 or 4 cells and their nuclei are generally in the resting state. In many of the Delesseriaceae and Ceramiaceae some of the cells of the carpogonial branches contain two or more nuclei. The vegetative cells in these forms are multinucleate, and it is not surprising that this nuclear condition should occur also in cells of the carpogonial branches. In a form like *Dumontia*, where nearly all the vegetative cells are uninucleate, it is surprising that any cells of the carpogonial branch should contain more than one nucleus. However, the cytoplasmic contents of the carpogonial cells are much greater than those of the adjoining vegetative cells in proportion to their size, and the presence of an extra amount of chromatin in the larger cells of the carpogonial branches is quite in accord with the current belief of a definite relation in volume between cell and nucleus. The mature carpogonium lies close to or in contact with the third cell (figs. 32–38).

Only 4 trichogynes with spermatia attached to them were found in all the material examined. These were found in the material collected in April 1915. Although this number is small, it is not less than would be expected, since only a very few trichogynes were found projecting beyond the surface of the thallus. This may have been due to the fact that the mature trichogynes persist for only a short time, or that they are easily broken off. It is to be regretted that none of these trichogynes with the spermatia attached to them could be traced back to the carpogonium. In none of these cases was it possible to find even the carpogonium. In one case one spermatium had fused with the tip of a trichogyne, while 7 others were merely adhering to its sides (fig. 41). Judging from the way it stained, the cytoplasm in this one spermatium and in the tip of the trichogyne had begun to disintegrate. The other spermatia stained very lightly, and it was not possible to

distinguish the structure of their contents. The cytoplasm in all the trichogynes with the spermatia attached to them appeared to be disintegrated, and no trace of a male nucleus was seen in any of them. Disintegrating cytoplasm stains very deeply in vegetative cells which have been injured, in trichogynes which have functioned, in carpogonial branches which have not been fertilized but are destined soon to disappear (fig. 36), and in those cells of the auxiliary cell apparatuses which are terminal and will also soon disappear. The cytoplasm of the trichogyne would not disintegrate as soon as the male nucleus had entered it, so that this nucleus in all these cases had probably passed into the carpogonium. Only one spermatium was attached to each of the other 3 trichogynes.

It has always been extremely difficult to obtain clear evidence concerning the phenomenon of fertilization in the Florideae. A few workers, as OLTMANNS (**9**), OSTERHOUT (**11**), HASSENCAMP (**3**), WOLFE (**19**), YAMANOUCHI (**20**), and SVEDELIUS (**16**) have succeeded in finding consecutive stages showing the fusion of the spermatium to the trichogyne, the passage of the male nucleus down the latter, and the fusion of the male and female nuclei in the carpogonium. The only two members of the Dumontiaceae in which the structure of the female reproductive organs has been carefully worked out are *Dudresnaya purpurifera* and *D. coccinea* (OLTMANNS **9**). OLTMANNS in *D. purpurifera* observed the entrance of the male nucleus into the trichogyne. The nucleus of the carpogonium at this time has moved out into the coiled portion of the trichogyne. No nucleus is present in the trichogyne in the next stage which he observed, but in the carpogonium there is a nucleus which he assumes to be the fusion nucleus. OLTMANNS states that he was not able to secure satisfactory evidence concerning the fusion of the male and female nuclei. He does not describe or picture fertilization in *Dudresnaya coccinea*, but states that it is in no way unusual. In *Dumontia* less evidence has been obtained concerning fertilization than OLTMANNS presented in the discussion of the two species of *Dudresnaya*. Nevertheless, there is really no reason to doubt the occurrence of fertilization in these forms.

As previously stated, the mature carpogonial branch is always bent around so that the carpogonium is close to or in actual contact

with the third cell. In many cases it lies very close to the second cell also. Thus the structure of the carpogonial branch suggests that the fusion nucleus passes from the carpogonium into the second or third cell. This evidently does occur, although satisfactory stages showing the process have not been found. Such figures as 42 and 43 show that the sporogenous filaments originate from either the second or third cells of the carpogonial branch. Since the actual passage of the fusion nucleus into the cell producing the sporogenous filaments has not been observed, there will naturally arise a question concerning the origin of the nuclei in these filaments. It cannot positively be stated that the nuclei in the sporogenous filaments are descended from the fusion nucleus of the carpogonium, but most of the evidence leads to this conclusion. Hundreds of carpogonial branches which have not been fertilized have been examined, and in only two or three cases is there any evidence that the third cell is binucleate. The second cell has never been observed to contain more than one nucleus. OLTMANNS (9) states that in *Dudresnaya coccinea* the cell of the carpogonial branch with which the sporogenous filament fuses is often binucleate, but that these nuclei never move out into the sporogenous filaments. Spermatia are found fused to trichogynes projecting beyond the surface of the thallus. Sporogenous filaments are found arising from cells of carpogonial branches whose trichogynes probably had projected beyond the surface of the thallus (fig. 42). The cells which produce the sporogenous filaments are those which in other carpogonial branches are always close to or in contact with the carpogonium. Considering these facts it seems highly probable in *Dumontia filiformis*, as in *Dudresnaya purpurifera* and *D. coccinea*, that the nuclei in the sporogenous filaments are derived from the fusion nucleus in the carpogonium. All the cells in the carpogonial branches stain very faintly at the time of the formation of the sporogenous filaments. The cytoplasm in all the cells, particularly the terminal ones, becomes very thin (fig. 42) and in some cases practically nothing but the cell walls is visible. The cytoplasm in these cells is disintegrating, but not in the same manner as it does in the trichogynes and some of the other cells. The failure to find the carpogonium may be due to the fact that it disintegrates

after it has discharged its nucleus. The carpogonium is much smaller than the cell which produces the sporogenous filaments, so that it might still be present, although not recognizable, after the fusion of the two cells.

The sporogenous filaments in *Dumontia*, according to SCHMITZ (**13**), grow out from the carpogonium and do not fuse with any cell in the carpogonial branch. This statement obviously is not correct. One cell of the carpogonial branch in *Dumontia* may produce three sporogenous filaments (fig. 42). A mass of fairly dense cytoplasm which always contains a nucleus and sometimes a chromatophore is present at the tip of each filament (fig. 42). The remainder of the filament appears to be entirely empty. The sporogenous filaments in *Dudresnaya purpurifera* (OLTMANNS **9**) arise from the carpogonium and do not fuse with any cell in the carpogonial branch. A carpogonial branch in *D. purpurifera* and *D. coccinea* produces 2 or 3 sporogenous filaments. Each of these filaments in *D. coccinea* fuses with a cell of the carpogonial branch before growing out into the tissue of the thallus. All the cytoplasm in the 3 sporogenous filaments in *D. purpurifera* is derived from the carpogonium, in *D. coccinea* from the carpogonium and 3 other cells of the carpogonial branch, and in *Dumontia* from either the second or third cell of the carpogonial branch.

AUXILIARY CELL BRANCHES

The auxiliary cell branches of *Dumontia* have the same origin and the same distribution as the carpogonial branches, but they are not so numerous as the latter. The ratio of carpogonial to auxiliary cell branches, considering the average number of branches initiated on a plant, is approximately 7 to 1. The carpogonial branches are very numerous in certain regions, as at the base of the mature cystocarpic plant. The auxiliary cell branches are found to predominate over the carpogonial branches at slightly higher level on this same plant.

The mature auxiliary cell branches vary in length from 4 to 6 cells (figs. 44, 45). The basal cell, just as in the carpogonial branch, may cut off a supernumerary cell (figs. 43, 45, 46). The size of the cells and the mode of development of the auxiliary cell branch

are essentially similar to those of the carpogonial branch. The youngest auxiliary cell apparatus which could be distinguished from a carpogonial branch consists of 3 cells (fig. 47). The similarity of the two branches is very apparent. The terminal cell of the auxiliary cell apparatus as a rule is not as pointed as that of the carpogonial branch (compare figs. 44–53 with figs. 24–28). There are, however, exceptions to this rule (figs. 48, 54). This cell may not be pointed even when it is about to divide (figs. 52, 53). There is also some difference in the way in which the cytoplasm of the cells of the two branches stains. This difference is so slight that it can be used as a criterion in distinguishing the two kinds of branches only when they are in one section or in sections which have been similarly fixed and stained. The basal cell of the auxiliary cell apparatus is often binucleate (fig. 50) and sometimes contains 3 nuclei (fig. 48), as does the similar cell in the carpogonial branch (fig. 36). None of the cells except the terminal one was ever observed to be binucleate in a carpogonial branch which was not mature. The auxiliary cell branch shown in fig. 49 is not mature, and the second cell is binucleate. Fig. 50 shows an immature branch in which 3 cells are binucleate. Some of the cells in the auxiliary cell branches contain chromatophores similar to those in the cells of the mature carpogonial branches and in the sporogenous filaments (figs. 44, 55). The auxiliary cell is either the second or third cell of the branch (figs. 43, 45, 57–60, 63). The sporogenous filament with the nucleus in its end grows toward the auxiliary cell branch (fig. 54).

The sporogenous filament fuses with the auxiliary cell (figs. 43, 45, 56, 59). Some of the cytoplasm of the sporogenous filament undoubtedly fuses with that of the auxiliary cell. This appears evident from the fact that the end of the sporogenous filament always contains cytoplasm and in some cases terminates in the auxiliary cell (figs. 45, 56, 59). After the fusion of the sporogenous filament with the auxiliary cell, the original nucleus of the latter maintains its former position (figs. 45, 60, 63) or withdraws to one side (fig. 58) as in *Dudresnaya purpurifera* and *D. coccinea*. It has been stated that cells 2 and 3 of the auxiliary cell branch are occasionally binucleate. This binucleate condition in the auxiliary

cell is apparently of no significance, because the nucleus from the sporogenous filament enters here just as it does in the uninucleate auxiliary cell (fig. 57). OLTMANNS reports that the sporogenous filaments in *Dudresnaya purpurifera* and *D. coccinea* branch freely. These filaments in *Dumontia* apparently branch only occasionally (figs. 42, 43). In both species of *Dudresnaya* no septa are formed in the filaments except when they fuse with the auxiliary cells. When the septa do occur, they are formed in the filament on both sides of its point of fusion with the auxiliary cell. The tip of the filament may then grow on to fuse with 2 or 3 more auxiliary cells. In *Dumontia* only one case was observed in which a filament has actually fused with an auxiliary cell and does not also terminate in the cell (fig. 43). No septa were seen in this filament. A few filaments growing over auxiliary cells were observed, but in these cases there was no indication of any fusion (figs. 44, 53). The sporogenous filament in fig. 43 branches just before it terminates in the auxiliary cell.

CYSTOCARPS

Carpospore development is initiated by the formation of 3 or 4 gonimoblast filaments, of about 3 cells each, which arise successively from the lateral protrusion of the auxiliary cell. These filaments branch once, often twice, and every cell forms a spore (figs. 56, 59). The cells at first are uninucleate (figs. 56, 58, 59, 63). At a little later stage they become binucleate and divide (fig. 62). No sterile cells are present at the base of the gonimoblast filaments. The carpospores when first formed are rounded or subangular and about 11 μ in diameter. They are well filled with a spongy cytoplasm which contains many small vacuoles (fig. 63). No chromatophores are visible, but often a number of small dark staining granules are present. When the nucleus is in the resting state, all the chromatin is in the nucleolus. In the young cystocarp there are generally present 3 or 4 cells of the auxiliary cell branch (figs. 56, 58, 63), and sometimes as many as 5 (fig. 62). A portion of the auxiliary cell branch is often present even in the mature cystocarp (fig. 60). The wall of the cystocarp is formed by branches which grow out from these subcortical cells that have been displaced inward by the enlargement of the group of carpospores (fig. 63).

The growth of these branches which form the pericarp is similar to that of the ordinary subcortical branches.

On an average 9 carpospores are present in a median transverse section of a mature cystocarp. Often 3 or 4 cystocarps will crowd together, so that in a section they appear as one. The average diameter of the mature carpospores is 38 μ. When the carpospores are actually mature, they are well filled with cytoplasm, contain a large amount of Floridean starch, and a number of protein granules. These granules respond to the stain and to the protein test in the same way as those in the tetrasporangia. These protein granules when they first appear are small and very numerous. In one carpospore in a median section 12 μ thick there are 170 of these granules (fig. 64). The ringlike chromatophores, about 2.5 μ in diameter, first appear in the carpospore just before it is mature. They are not peripheral but are scattered throughout the entire protoplast. Chromatophores are often present in the sporogenous filaments and in the auxiliary cells, but have never been seen in the latter at the time the carpospores are formed. It is possible that chromatophores which do not take the stain are present in these cells, although it seems hardly probable that they could be completely overlooked, since the cytoplasm in the auxiliary cell is very thin and much vacuolated. It is generally believed that chromatophores never arise *de novo*, and SCHMITZ (**12**) has stated that they are always present in the spores of the Florideae. In other Florideae besides *Dumontia* the chromatophores are evidently not readily seen at this stage, since their presence in the young carpospores is rarely mentioned.

The protein granules in the mature carpospores often disappear just before the spores are discharged, and are never present in the germinating spores. Certain of the chromatophores increase greatly in their staining power coincident with the disappearance of these granules (fig. 65). There has evidently been some modification in the substance of these chromatophores, and it seems quite possible that the substance of the protein body is concerned with this change. The chromatophores in the mature carpospores which have thus become differentiated stain with the same intensity as those in the germinating carpospores and appear to have the same

structure as those in the mature tetraspores. The majority of carpospores in a mature cystocarp contain one large nucleus each (fig. 66). Occasionally a spore which is just about to escape contains 2 nuclei (fig. 67). The spore shown in fig. 67 was directly behind a spore which was just passing through the pore in the wall of the cystocarp. The fact that the nucleus divides in some of these carpospores just as they are escaping indicates that the spores germinate as soon as they are discharged. In fact, the spores sometimes germinate while still inclosed in the cystocarp. In most of the female plants collected, the tips of the main axis of the thallus and branches were frayed out. The mature carpospores are present at these points, and it is therefore evident that the disintegration of the cells surrounding them furnishes one possible means of escape. As the carpospores enlarge, they compress the surrounding vegetative cells on all sides, and also cause the wall of the thallus to bulge out. The layers of cortical and subcortical cells gradually become thinner on the bulging side of the pericarp, until finally they are ruptured and the naked carpospores escape through the pore thus formed (fig. 68, 1).

Groups of multinucleate cells, which are of the same size and have the same position as the normal cystocarps, occasionally occur in the wall of the thallus. In group 1, fig. 68, a section of a normal cystocarp, 16 spores appear to be present, but probably not all of these are in this one cystocarp. Similar sections of two groups of multinucleate cells on the other side of the thallus (2 and 3) contain respectively 30 and 70 cells. Some of the cells in the groups of multinucleate cells are uninucleate and of the same size as the mature carpospores (fig. 66), while others of approximately the same size or smaller contain 2 or 3 nuclei (figs. 67, 69, 71). In some cases nuclear division is followed by cell division (fig. 70). Evidently after one of these larger spores divides, the daughters may in turn become multinucleate (fig. 71). From the arrangement of some of the cells it appears as though the larger cells have divided to form the smaller ones. The number of nuclei in the cells of a cystocarp similar to group 3, fig. 86, does not seem to be determined by the size of the cells. Some of the smaller cells may contain as many as 11 nuclei and the larger ones only

1 or 2. These are certainly nuclei and not pyrenoids, since they were clearly distinguished by hematoxylin, safranin, or methyl green. Many of these cells are somewhat vacuolated, and none of them contains protein granules or visible chromatophores. No cases have been observed in which any of these cells are escaping from the cavity. It seems probable from all the evidence available that such a group as 3 is formed by division of the spores of a normal cystocarp, and 2 is an intermediate stage between 1 and 3. Each of these groups of multinucleate cells, therefore, is the product of an abnormal cystocarp.

Germination of the carpospores may begin long before they escape from the thallus. The first step in germination is the formation of a gelatinous wall 2 μ thick (fig. 72). The chromatophores in these spores were 3 μ in diameter and stained darkly. They are similar in structure to those in the tetraspore, but are larger and more openly clathrate (figs. 73, 74). The chromatophores are not merely peripheral, but, as in the younger carpospores, are scattered throughout the whole protoplast. The next step in germination is the elongation of the spore until it becomes somewhat pear-shaped (fig. 74). The nucleus then divides and the first cell wall is formed perpendicular to the longitudinal axis of the carpospore (fig. 75). The narrow cell, as in the germinating spores of *Fucus*, is destined to form the basal part of the young plant. Neither growth nor cell division takes place as rapidly here as in the upper cells. The next wall formed apparently divides the upper cell obliquely (fig. 76). In a longitudinal section of an older sporeling these two upper cells were divided into 9 cells and the lower cell into 3 cells. All the cells of these germinating carpospores are rich in cytoplasm and contain chromatophores. The maximum size of the sporelings examined was 235 μ by 123 μ. In cavities containing germinating carpospores traces of disintegrating cytoplasm and nuclei have been observed, showing that some of the unicellular carpospores have degenerated.

CYTOLOGY

The nuclei in the auxiliary cell and the carpogonial branches are the most satisfactory ones in the cystocarpic plants for the study of

mitosis, since they are considerably larger and divide more actively than the vegetative nuclei. The cell history of these branches is also of some aid in identifying stages in nuclear division. All the chromatin in the nuclei of most of the young carpogonial branches is in the nucleolus (figs. 22–30). This is true of the nuclei also in cells 4, 5, and 6 of the mature branches (figs. 32–34, 37). The nuclei in cells 1, 2, and 3 of the mature carpogonial branches have a tendency to divide. The failure to secure any stages of mitosis in the nuclei of the cells of the young carpogonial branches is probably due to the fact that these cells divide very rapidly. The chromatin in the nuclei in most of the uninucleate cells of the mature auxiliary cell branches is not in the nucleolus but in a number of small granules (figs. 48, 51–55). The nuclei in the cells of these branches divide often (figs. 46, 48–50). The frequency of division of these nuclei is probably due, as in the basal cells of the carpogonial branch, to the fact that these cells are usually completely filled with dense cytoplasm (fig. 51). Thus in the resting nuclei of the cells of the carpogonial branches, as in the tetraspores and vegetative cells, all of the chromatin is in the nucleoli.

The following changes are observed in the nuclei in preparation for division. Radial fibrillae appear running from the nucleolus to the nuclear membrane (cell 2, fig. 55). Small chromatin granules pass out from the nucleolus, along the fibrillae, to the nuclear membrane. When the granules first appear on the linin strands, there is no appreciable decrease in the size of the nucleolus. The position of these granules when they first appear indicates that they have come from the nucleolus. Of the 6 granules present in fig. 77, 3 are in contact with the nucleolus, and only 1 has yet reached the periphery of the nucleolus. Nearly all of the granules at a slightly later stage are present only at the points where the fibrillae terminate in the nuclear membrane (fig. 78). More linin strands are formed which connect the radial fibrillae already present (fig. 79). All of the chromatin evidently passes out of the nucleolus and becomes distributed along the linin net. The net disappears just before the nucleus divides (fig. 80). Practically all of the chromatin in the nuclei which have just divided is in 7 fairly uniform granules (cell 4, fig. 52). The nucleus of cell 4,

fig. 53, is evidently just dividing. All of the chromatin in the nucleus of this cell is in 14 granules of approximately the same size. Nuclei which are probably preparing for division often contain 7 similar chromatin bodies (cell 3, fig. 51; cell 4, fig. 55; fig. 81). It is thought that the chromatin bodies in these nuclei may be chromosomes. Nuclei in the earlier stages of division contain 16–24 granules (cells 1 and 3, fig. 52; figs. 79, 80). These granules must become grouped together to form the chromosomes. Thus possibly the haploid number of chromosomes in *Dumontia* is 7. However, it is evident that not enough data have been accumulated to determine with any degree of certainty the number of chromosomes. These larger chromatin bodies in some of the nuclei are vacuolated (cell 3, fig. 51). All the chromatin in the resting nucleus is in the nucleolus, hence the chromosomes must fuse together after division. It is quite possible that all the chromosomes do not fuse at one time. Thus in fig. 82 each of the 2 large chromatin bodies in the nucleus which contains 5 may have been formed by 2 chromosomes fusing together. If the fusing continued, the nucleus would appear quite similar to that in the adjoining cell. When the nucleus is being organized after division, the nucleolus appears granular, and a few small chromatin bodies may, for a time, remain outside of it (fig. 62). In the nucleus of the mature carpospore the linin net is well developed, and all the chromatin is in the nucleolus, which always contains at least one vacuole (fig. 66). Our knowledge of the details of mitosis in this species of *Dumontia* is as yet very fragmentary. The stage represented in cell 3, fig. 52, is similar to the prophase of *Delesseria* as described by SVEDELIUS (15). This cannot be the prophase in *Dumontia* because the granules present at this stage collect together to form larger units, probably chromosomes.

In *Polysiphonia* (YAMANOUCHI 20) and *Delesseria* (SVEDELIUS 15) the chromatin from which the chromosomes are formed is never contained in the nucleolus. It is distributed in fine granules along the linin threads. The granules are in groups or short rows, each one of which represents a prochromosome. A chromosome is then formed by the fusion of several granules. Mitosis in *Dumontia* up to the time of chromosome formation seems to be

exactly similar to that in *Nemalion* (WOLFE **19**). Nearly all the chromatin in the resting nucleus of *Dumontia* is in the nucleolus, and, as in *Nemalion* and *Griffithsia* (LEWIS **6**), this chromatin passes out along the fibrillae to the periphery of the nucleus. The number of granules present in *Griffithsia* and *Nemalion* seems to be about twice the number of chromosomes formed. This may be the case in *Dumontia* also, although in this form the number of granules seems proportionately larger. There is no indication of any chromatin being expelled from the nucleus of *Dumontia* as it is in *Griffithsia*.

Discussion and results

The auxiliary cell branch and carpogonial branch of *Dumontia filiformis* resemble each other very closely in origin, mode of development, and structure. This similarity is so great that in some cases it is almost impossible to determine the character of a branch. The number, arrangement, and contents of the cells may be the same in these two kinds of branches. The trichogyne persists for only a short time after it has functioned. Hence the absence of this structure is not a safe criterion for distinguishing the auxiliary cell branches. The carpogonial and auxiliary cell branches differ greatly from the vegetative branches in the size and contents of their cells. It seems quite possible that the auxiliary cell branches in *Dumontia* once bore trichogynes and functioned as carpogonial branches. This similarity in structure of the auxiliary cell and carpogonial branches is almost as marked in *Dumontia* as in *Dudresnaya coccinea*. The auxiliary cell branch of *D. coccinea* consists of 12 cells and the carpogonial branch of 7 cells (OLTMANNS **9**); otherwise the two kinds of branches appear similar in origin and structure and differ greatly from the vegetative branches.

It has been stated that the auxiliary cell branches and carpogonial branches of *Dumontia* are probably homologous structures. If this is true, the sporogenous filaments were probably developed at the time when certain carpogonial branches ceased to be capable of fertilization. The male plants in this species of *Dumontia* are present for only 2 or 3 weeks during each spring. The ratio of the number of female to male plants at the time when the latter are supposed to be at the height of their development is 3 to 1.

An examination of scores of young female plants has shown that the trichogynes must persist for only a short time after they have reached the surface of the thallus. Under these conditions an arrangement whereby the fertilization of one carpogonium would make possible the development of 3–6 cystocarps would evidently be of considerable advantage to the plant. If the auxiliary cell branches are carpogonial branches which have ceased to function, *Dumontia* presents a case quite parallel to that of *Corallina*. In *Corallina* (OLTMANNS 10) only those carpogonia in the center of the conceptacle which bear long trichogynes are capable of being fertilized. The carpogonia at the periphery of the conceptacle cannot be fertilized because they bear no trichogynes. In each conceptacle often only one carpogonium is fertilized. Descendants of the fusion nucleus in this one carpogonium pass to the auxiliary cells of many procarps, and several cystocarps develop in one conceptacle.

Cell 2 or 3 of the carpogonial branch of *Dumontia* probably functioned as the auxiliary cell before the plant had acquired the habit of forming sporogenous filaments. It is cell 2 or 3 of the auxiliary cell branch which forms the carpospores. One of the 3 cells in the carpogonial branch of *Dudresnaya coccinea* with which the sporogenous filaments fuse, before passing to the auxiliary cell branches, at one time probably functioned as the auxiliary cell. The sporogenous filament often fuses with the fifth cell of the carpogonial branch and with the fifth cell of the auxiliary cell branch. In both *Dumontia* and *Dudresnaya coccinea*, therefore, that which is supposed to have been the original auxiliary cell and that which now functions as such occupy similar places in their respective branches. The families of the Cryptonemiales show a considerable variation in the distribution and structure of their auxiliary cell and carpogonial branches. Even the species in one genus as *Dudresnaya* (OLTMANNS 9) may vary greatly in this respect. It would then be rather surprising to find that the history of the development of the auxiliary cells in all the Cryptonemiales is similar. OLTMANNS (9) suggests that the sporogenous filaments of the Cryptonemiales have been developed from the gonimoblast filaments of such forms as *Wrangelia* and *Naccaria*. He considers

Nemastoma the transitional form between the Nemalionales and the Cryptonemiales. The auxiliary cells of *Nemastoma* do not occur in special branches, but are modified cells of the cortical hyphae. Thus in *Nemastoma* there are no auxiliary cell branches which can be considered as homologous with the carpogonial branches. But there is also the evidence which has been presented that the auxiliary cell branches in some forms as in *Dumontia* are not vegetative hyphae which have by chance become highly specialized in the same manner as the carpogonial branches. Thus the sporogenous filaments, structures which are peculiar to this one order, the Cryptonemiales, have probably been developed along two independent lines.

The female reproductive organs of the other red algae are relatively simple when compared with those of the Cryptonemiales. It is not surprising that the history of the nuclei in the sporogenous filaments and auxiliary cells of the members of this order was an especially puzzling problem to the earlier students. It has been stated in the introduction to this paper that the origin of the nucleus functioning in the auxiliary cells at the time of the formation of the carpospores proved to be a stumbling block to most of these students. The results of the work of SCHMITZ on certain genera of the Cryptonemiales, including *Dudresnaya*, *Dumontia*, and *Gloeosiphonia*, were conflicting in regard to the occurrence of a fusion between the sporogenous and auxiliary cell nuclei. OLTMANNS' (**9**) investigation established practically beyond doubt the two following facts: the sporogenous and auxiliary cell nuclei in *Dudresnaya* and *Glocosiphonia* do not fuse, and the nuclei in the carpospores are descended from the sporogenous nucleus and not from the original auxiliary cell nucleus.

No member of the Cryptonemiales has been carefully investigated since 1898 in regard to the occurrence of a nuclear fusion in the auxiliary cell. A considerable amount of excellent work, however, has been done on other red algae during the last 17 years. The fusion nucleus or one of its daughters, in many genera, has been traced from the carpogonium into the auxiliary cell. The fusion nucleus in all these forms appears to take charge of the cytoplasm in the auxiliary cell and becomes the ancestor of the nuclei

in the carpospores. In none of these forms is the nucleus in the auxiliary cell reported to fuse with the nucleus entering it from the carpogonium. Thus HASSENCAMP (3), YAMANOUCHI (20), SVEDELIUS (14), and LEWIS (6) have found, in the forms studied by them, that only one nuclear fusion occurs during fertilization and the formation of the carpospores, and this is the fusion between the nucleus of the spermatium and that of the carpogonium. It would seem that the evidence is overwhelming against the occurrence of a second fusion in the auxiliary cell. Undoubtedly one reason why OLTMANNS' work has been questioned is the fact that certain morphologists have for years cherished the theory that a relationship could be established between the Ascomycetes and the Florideae. The ascogonium of certain genera is remarkably similar in structure to the carpogonium of some of the Florideae. No other plants except those belonging to these two classes have this kind of a female reproductive organ. According to some workers, certain genera of the Ascomycetes are distinguished from all other plants by the fact that two distinct nuclear fusions occur during fertilization and the formation of the spores. If it could be shown that a second nuclear fusion does actually occur in the auxiliary cell of such a form as *Dudresnaya* or *Dumontia*, the carpogonium with its trichogyne and long sporogenous filaments with the carpospores at their extremities might be proved to be homologous with the ascogonium of some form like *Pyronema* with its trichogyne and long ascogenous hyphae bearing ascospores.

It has been stated that in all probability the nuclei in the sporogenous filaments of *Dumontia* are descended from the fusion nucleus in the carpogonium. However, more evidence is to be desired in regard to the origin of these nuclei. The sporogenous nuclei in *Dudresnaya purpurifera*, *D. coccinea*, and *Gloeosiphonia* (OLTMANNS 9) are unquestionably derived from the fusion nucleus in the carpogonium. In *Dumontia*, as in the 3 species of the Cryptonemiales studied by OLTMANNS, there can be no doubt in regard to the passage of a nucleus from a sporogenous filament into an auxiliary cell. A nucleus is always present in *Dumontia* at the tip of each filament. Tips of filaments are found lying quite near the auxiliary cells. A similar filament is found fused to the auxiliary

cell, and no nucleus is then present in the filament. There are, however, two widely separated nuclei in the auxiliary cell itself, and one of these lies quite near the point of fusion of the filament and the cell. The sporogenous nucleus in *Dudresnaya purpurifera*, *D. coccinea*, and *Dumontia filiformis* at no time even closely approaches the original auxiliary cell nucleus. The 2 nuclei in all 3 species lie at almost opposite ends of the cell. The carpospores are budded off from that end of the auxiliary cell which contains the sporogenous nucleus. In *Dumontia* the original auxiliary cell nucleus and the descendant of the sporogenous nucleus could be identified in nearly all the auxiliary cells which bore carpospores. OLTMANNS (9) observed in *Gloeosiphonia* and *Dudresnaya* several cases of "blind fusion" where, although a sporogenous filament had fused with an auxiliary cell, no nucleus had passed over and the auxiliary cell contained only its own nucleus. No examples of "blind fusion" were found in *Dumontia*. It would seem that OLTMANNS' statement that it is a daughter of the sporogenous nucleus in *Gloeosiphonia* which moves into the pericentral cell might be questioned. The two daughters of the auxiliary cell nucleus and the sporogenous nucleus always lie close together. One of these 3 nuclei divides and one of the daughters moves into the pericentral cell. In *Dudresnaya purpurifera*, *D. coccinea*, and *Dumontia* there can be no question as to the origin of the nuclei in the carpospore. They are derived from the sporogenous nucleus.

Summary

Dumontia filiformis, during May, June, and the first half of July, grows in abundance in the tide pools and on the bed rock at South Harpswell, Maine. This alga became established on the coast at South Harpswell between 1905 and 1913. Antheridial, cystocarpic, and tetrasporic plants may have essentially identical size and vegetative structure. The average size of the antheridial plants is a little less than that of the other plants. Cystocarpic and tetrasporic plants are found growing together on the same rock and in the same tide pools. Female plants which bear mature cystocarps are easily recognized by the protrusions which these form in the wall of the thallus. The type of branching varies

considerably. All male and female plants collected were branched. Tetrasporic plants, simple and branched, were found. The maximum number of branches observed on any individual plant was 30. The color of the plants varies from dark red to pale reddish yellow. Male plants bearing mature spermatia are present in the early part of April and two weeks later have almost completely disappeared. Young female plants were found on April 12, and these reach their maximum stage of development about the middle of May. Tetrasporic plants are most abundant in the latter part of June and have almost entirely disappeared by the first of August. *Dumontia* at South Harpswell must persist through the winter in the form of sporelings developed from the tetraspores.

The vegetative structure is of a type occurring in many families of the Florideae. The disk-shaped holdfast is composed of a single layer of horizontal filaments, each cell of which produces a vertical ascending branch. Certain of these branches elongate to form the medullary hyphae of the tubular thallus. Each medullary hypha has its own initial cell. Every cell of each medullary hypha produces a radial branch. These radial branches, by repeated dichotomous forking, form the subcortex and the cortex. Growth is apical throughout the entire thallus. All the other vegetative cells, except the trichomes, are uninucleate and contain one chromatophore each. All the chromatin in the resting nucleus is in the nucleolus. The chromatophore is a clathrate hollow ellipsoid, lying just inside the cell wall.

The tetraspores are imbedded in the wall of the thallus, and are distributed evenly throughout practically the entire length and circumference of the branches and main axis. Younger tetrasporangia are found toward the base of the plant. The larger subcortical cells become modified to form the tetrasporangia. The chromatophore becomes constricted at intervals, so that it appears to consist of rows of small irregular plates. These bodies persist through all stages of the tetrasporangium, and their number is increased in the tetraspores. No spindle or spireme was seen. The chromatin in some of the nuclei is in several small bodies, but these do not resemble chromosomes. The first cleavage furrow completely divides the tetrasporangium, is perpendicular

to its longitudinal axis, and is parallel to the surface of the thallus. The chromatophores in the tetraspores are hollow, oval bodies with perforated walls. The mature tetraspores do not round off while imbedded in the thallus. They escape either by the disintegration of the cells surrounding them or by a pore formed in the wall of the thallus.

The spermatia form a continuous layer over nearly the entire surface of the thallus. The spermatium mother cells terminate the branches forming the cortex and subcortex. The chromatophore which is present in the young spermatium mother cell partially or completely disappears as this cell matures. The protoplasm which was in the chromatophore is used in forming the granular cytoplasm of the mature cell. The youngest spermatium mother cells observed were binucleate. The first spermatium is cut off diagonally. The mother cell may again become binucleate and cut off a second spermatium in a similar manner on the side opposite to that on which the first was formed. The second spermatium may be formed while the first one is still attached to the mother cell. No chromatophore is present in the spermatium. The nucleus is situated at the distal end of the spermatium in a dense mass of cytoplasm. The proximal end is vacuolated. The spermatium is cut off from the mother cell as a cell and not as a naked protoplast.

The distribution of carpogonial branches in the young female plants is general, as in the case of the tetrasporangia in the tetrasporic plants. The carpogonial branch develops by apical growth and arises as a lateral outgrowth of a large subcortical cell. A mature carpogonial branch consists of 6 or 7 cells and a trichogyne (6 cells always lie in a row). The basal cell ("cell 1") sometimes divides to form a lateral cell. The carpogonium in a mature branch is always close to or in contact with "cell 2" or "cell 3." The sporogenous filaments always arise from one of these two cells. Only a few trichogynes were found projecting beyond the surface of the thallus. Spermatia were found fused to 4 trichogynes.

Each carpogonial branch which has been fertilized produces 2 or 3 sporogenous filaments, all of which arise from one cell. It is thought that the nuclei in these filaments are descended from

the fusion nucleus in the carpogonium. The sporogenous filaments grow out toward the auxiliary cell branches. The auxiliary cell branches in origin, distribution, structure, and mode of development are very similar to the carpogonial branches. Only about 1 auxiliary cell branch is initiated to every 7 carpogonial branches. The time of initiation of the former is a little later than that of the latter. The mature auxiliary cell branch consists of 4–7 cells. The second or third cell of the branch is the auxiliary cell, the cell with which the sporogenous filament fuses and the one which forms the carpospore. The original nucleus in the auxiliary cell takes no part in the formation of the carpospores. The nuclei in the carpospores are descended from the nucleus which enters the auxiliary cell from the sporogenous filament.

In the development of the carpospores and cystocarps 3 or 4 gonimoblast filaments arise from the auxiliary cell. Every cell of these filaments forms a spore. There are about 20 carpospores in each cystocarp. The pericarp is formed by radial branches similar to those which form the subcortex and cortex of the wall of the thallus. Mature carpospores are usually uninucleate, well filled with a cytoplasm, and contain chromatophores. The chromatophores are similar to those of the vegetative cells. The nucleus sometimes divides just as the carpospore is about to escape. Naked carpospores escape through a pore formed in the pericarp. Carpospores sometime germinate while in the cystocarp. The ends of branches of mature plants fray, and this disintegration of cells surrounding the cystocarp furnishes one means of escape for the carpospores and sporelings.

In the resting nucleus of *Dumontia* all the chromatin is in the nucleolus. The nucleolus often contains a vacuole. The chromatin in preparation for mitosis passes out of the nucleolus and in the form of small granules becomes distributed along the linin net. The net disappears and the granules become massed together to form larger units, chromosomes. The number of chromosomes was not definitely determined, but was apparently about 7. No spireme or spindle was seen. After division, the chromatin is again found massed together in the nucleolus.

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EXPLANATION OF PLATES XIX–XXII

Lettering of figures.—*aux.n.*, auxiliary cell nucleus; *chr.*, chromatophore; *cps.*, carpospores; *m.h.*, medullary hyphae; *p.g.*, protein granule; *sbc.c.*, subcortical cell; *s.c.*, supernumerary cell; *sp.fil.*, sporogenous filament; *sp.n.*,

sporogenous nucleus; *spm.m.c.*, spermatium mother cell; *st.c.*, stalk cell; *tr.*, trichogyne.

PLATE XIX

FIG. 8.—Vertical section through holdfast showing horizontal hypha and vertical ascending, dichotomously branched hyphae; ×355.

FIG. 9.—Slightly diagrammatic; vertical section through base of plant and holdfast; ×170.

FIG. 10.—Longitudinal section through wall of thallus showing radial hyphae arising as branches of longitudinal, medullary hyphae; ×700.

FIG. 11.—Longitudinal section through apex of young branch; ×340.

FIG. 12.—Surface view of subcortical cell showing structure of chromatophore; ×1000.

FIG. 13.—Longitudinal section through an intercellular connection showing granules of which disk is composed; ×700.

FIG. 14.—Longitudinal section through an intercellular connection, between 2 carpospores, in which granules have not yet become grouped together to form disks; ×1000.

FIG. 15.—Transverse section through wall of thallus of male plant showing stalk cells, spermatium mother cells, and spermatia; ×1000.

FIG. 16.—Longitudinal section through binucleate spermatium mother cell; ×1400.

FIG. 17.—Similar section; spermatium mother cell which has budded off one spermatium; ×1400.

FIG. 18.—Transverse section through wall of male plant showing free spermatia imbedded in gelatinous sheath of thallus; ×1000.

FIG. 19.—Longitudinal section through spermatium mother cell and spermatium; chromatophore at base of spermatium mother cell; ×1400.

FIG. 20.—Longitudinal section through wall of thallus showing origin of carpogonial branches; ×355.

FIGS. 21, 22.—Longitudinal section of first cell of carpogonial branch, showing mode of origin from subcortical cell; chromatophore is similar to those of vegetative cells; ×1000.

FIG. 23.—Longitudinal section of a 2-celled carpogonial branch; ×1000.

FIG. 24.—Similar section of a 3-celled carpogonial branch; ×1000.

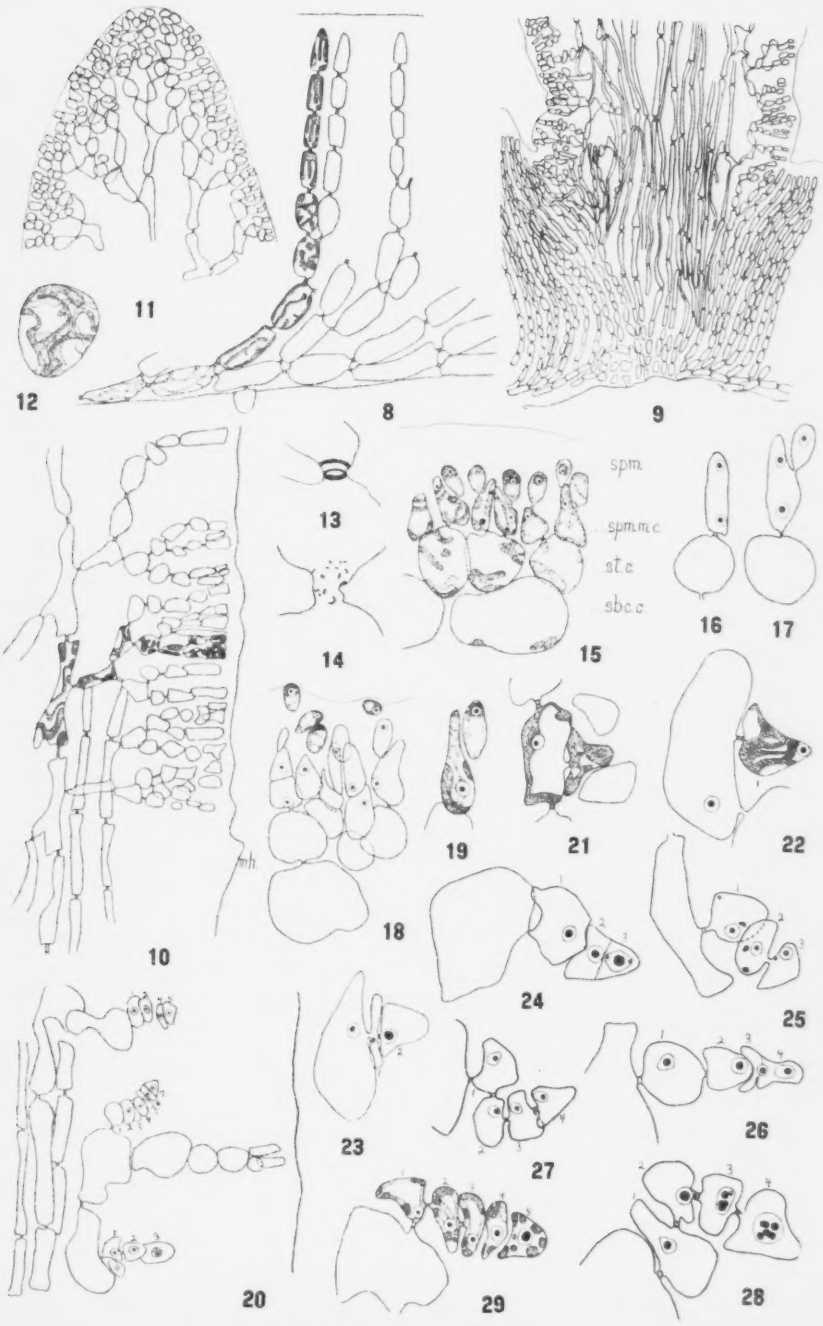
FIG. 25.—Longitudinal section of a 3-celled carpogonial branch; ×1000.

FIG. 26.—Similar section of a 4-celled carpogonial branch showing that number of cells is increased by division of terminal cell; ×1000.

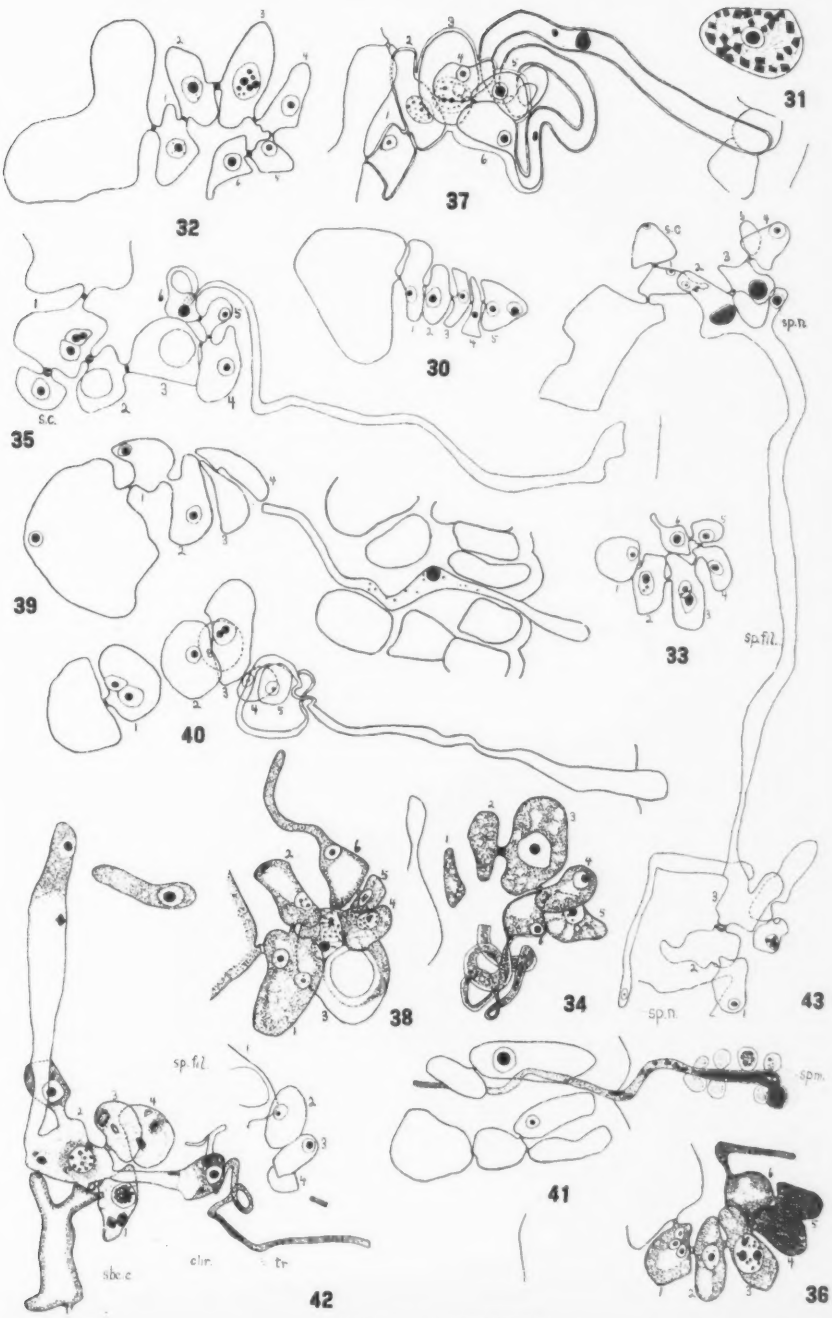
FIG. 27.—Similar section of a 4-celled carpogonial branch showing that branches at this stage may be bent to form a right angle; ×1000.

FIG. 28.—Four-celled carpogonial branch; nucleus in terminal cell contains 7 chromosomes (?); ×1000.

FIG. 29.—Five-celled carpogonial branch showing position and structure of chromatophores; ×1000.



DUNN on DUMONTIA



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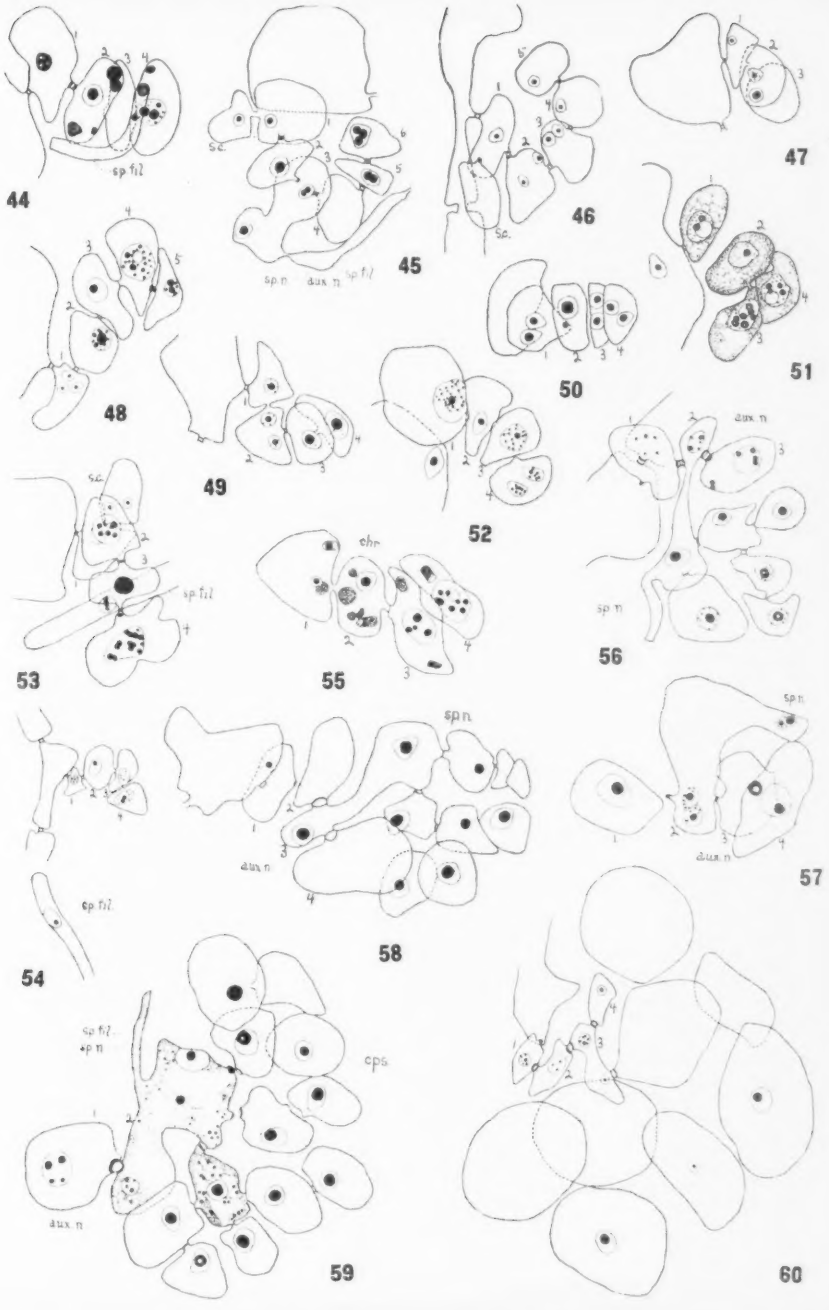

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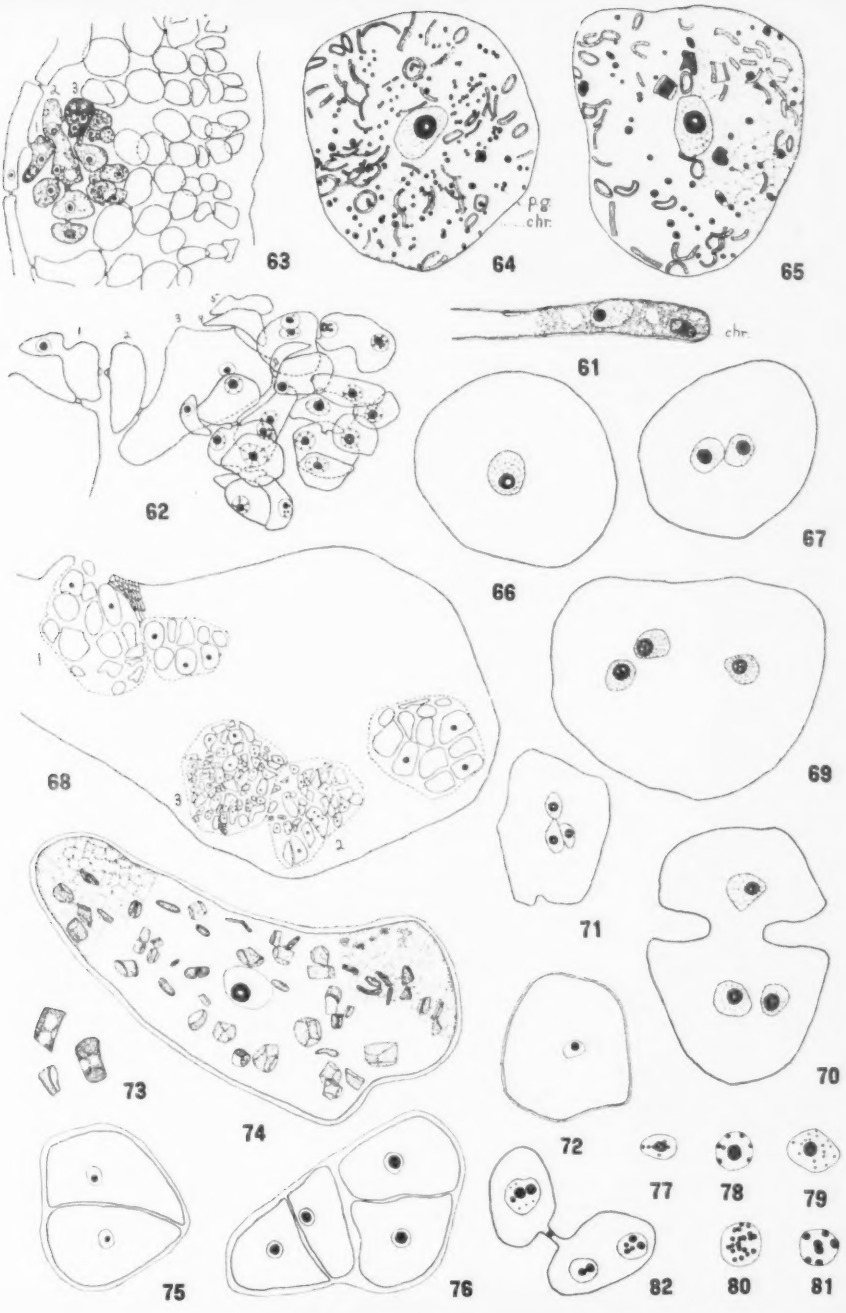

DUNN on DUMONTIA

PLATE XX

FIG. 30.—Similar carpogonial branch; terminal cells binucleate; ×1000.

FIG. 31.—Section of fifth cell of a 6-celled carpogonial branch; 26 chromatophores distributed on the cytoplasmic net; ×1400.

FIG. 32.—Six-celled mature carpogonial branch showing carpogonium lying near cell 3; ×1000.

FIG. 33.—Similar to fig. 32; cell 3 is binucleate; ×1000.

FIG. 34.—Six-celled carpogonial branch showing coiled trichogyne; ×1000.

FIG. 35.—Seven-celled carpogonial branch showing trichogyne reaching almost to surface of thallus; ×1000.

FIG. 36.—Six-celled carpogonial branch showing carpogonium in contact with cell 3; cell 1 contains 3 nuclei; cytoplasm in cells 4, 5, and 6 is beginning to disintegrate; ×1000.

FIG. 37.—Six-celled carpogonial branch showing much coiled trichogyne reaching almost to surface of thallus; ×1000.

FIG. 38.—Six-celled carpogonial branch showing carpogonium in contact with cell 3 which is much enlarged, nucleus of which is preparing to divide; ×1000.

FIGS. 39, 40.—Base of carpogonial branch, and trichogyne projecting beyond surface of thallus; ×1000.

FIG. 41.—Base of carpogonial branch (cells 1–4), and trichogyne projecting beyond surface of thallus; 8 spermatia attached to trichogyne; ×1000.

FIG. 42.—Carpogonial branch; 3 sporogenous filaments arising from cell 2; ×1000.

FIG. 43.—Carpogonial branch and auxiliary cell branch connected by sporogenous filaments; auxiliary cell branch consists of 6 cells; ×700.

PLATE XXI

FIG. 44.—Auxiliary cell branch; sporogenous filaments lying over cell 4; ×1000.

FIG. 45.—Auxiliary cell branch; sporogenous filament fused with cell 3; ×1000.

FIG. 46.—Auxiliary cell branch; cells 2 and 3 binucleate and a supernumerary cell present; ×1000.

FIG. 47.—Three-celled auxiliary cell branch; ×1000.

FIG. 48.—Five-celled auxiliary cell branch; nucleus of cell 4 preparing to divide; cell 1 contains 3 nuclei; ×1000.

FIG. 49.—Auxiliary cell branch consists of 4 cells; cell 2 binucleate; ×1000.

FIG. 50.—Four-celled auxiliary cell branch; cells 1, 3, and 4 binucleate; ×1000.

FIG. 51.—Four-celled auxiliary cell branch showing dense cytoplasmic contents of cells; 7 chromosomes and colorless nucleolus in nucleus of cell 3; ×1000.

FIG. 52.—Four-celled auxiliary cell branch; part of chromatin in nuclei of cells 1 and 3 in granules distributed on linin net; cell 4 contains 2 nuclei; ×1000.

FIG. 53.—Four-celled auxiliary cell branch; sporogenous filaments lying under cell 3; ×1000.

FIG. 54.—Sporogenous filament lying near 4-celled auxiliary cell branch; ×550.

FIG. 55.—Auxiliary cell branch showing structure of chromatophores; ×1000.

FIG. 56.—Auxiliary cell branch; 5 carpospores budded off from auxiliary cell; sporogenous filament fused with auxiliary cell; ×1000.

FIG. 57.—Auxiliary cell branch; auxiliary nucleus has divided; sporogenous nucleus has just entered auxiliary cell; ×1000.

FIG. 58.—Section through auxiliary cell branch and 8 young carpospores; auxiliary nucleus and sporogenous nucleus at opposite ends of auxiliary cells; ×1000.

FIG. 59.—Section through auxiliary cell branch and group of young carpospores; auxiliary cell and one carpospore shaded; sporogenous filament fused with auxiliary cell; ×1000.

FIG. 60.—Section through auxiliary cell branch and group of mature carpospores; auxiliary nucleus and sporogenous nucleus at opposite ends of auxiliary cell; ×550.

PLATE XXII

FIG. 61.—Same sporogenous filament as in fig. 75 showing cytoplasm nucleus and chromatophore at tip; ×1000.

FIG. 62.—Section through young cystocarp; 2 of the 18 carpospores which have arisen from auxiliary cells binucleate; ×1000.

FIG. 63.—Section through young cystocarp showing origin of radial branches which will form pericarp; ×350.

FIGS. 64, 65.—Partly shaded; sections of mature carpospores showing structure of nuclei, distribution of chromatophores, and protein granules; ×1000.

FIGS. 66, 67.—Sections of carpospores in a mature cystocarp; spores of same size and exactly similar to those which occur in abnormal cystocarps; ×700.

FIG. 68.—Transverse section of thallus showing 3 normal and 2 abnormal cystocarps; ×350.

FIGS. 69, 70, 71.—Sections through carpospores in abnormal cystocarps showing that nuclear division is not always directly followed by cell division; ×700.

FIG. 72.—Section of germinating carpospore showing cell wall; ×170.

FIG. 73.—Chromatophores of germinating carpospores; ×1400.

FIG. 74.—Partly shaded; longitudinal section of germinating carpospore showing structure and distribution of chromatophores and structure of cytoplasm; ×1000.

FIGS. 75, 76.—Sections of germinating carpospores showing sequence of cell walls during germination; ×170.

FIG. 77.—Nucleus of cell 3 of auxiliary cell branch showing chromatin granules passing from nucleolus to periphery of nucleus; ×1400.

FIG. 78.—Nucleus of cell 3 of auxiliary cell branch showing chromatin granules at periphery of nucleus; ×1400.

FIG. 79.—Nucleus of cell 3 of a carpogonial branch; small chromatin granules present on linin net; ×1400.

FIG. 80.—Nucleus of cell of an auxiliary cell branch; chromatin in small granules; no nucleolus or linin net present; ×1400.

FIG. 81.—Nucleus of cell 1 of a carpogonial branch showing 7 bodies which may be chromosomes; ×1400.

FIG. 82.—Section of 2 cells of an auxiliary cell branch showing chromatin granules in nuclei; ×1000.

PERMEABILITY OF MEMBRANES AS RELATED TO THEIR COMPOSITION

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 231

F. E. DENNY

(WITH SIX FIGURES)

Introduction

In a previous paper (1) measurements were made of the rate at which water passes through known areas of certain membranes. Different species of plants and different membranes of the same species showed large differences in the rate of penetration. From these facts the following questions arise. What substances in the membrane are determining the rate at which water can pass through it? Of what relative importance in this process are lipoids, proteins, tannoids, suberin, pectic substances, etc.? An attempt was made to answer these questions by quantitative measurements, and this paper records the results.

The rôle of different substances in the membrane in regulating its permeability to water was determined by comparing the permeability of a membrane before and after extracting it with the solvent of the material studied. Thus, for example, to determine the effect of lipoid materials upon the permeability of the membrane, its permeability in the normal condition was first measured; it was then extracted with a lipoid solvent (for example, acetone), and its permeability again measured. These two measurements were then compared. To accompany these measurements, microchemical tests were made to give information as to the nature of the materials composing the membrane, and the effect of these extractions upon its composition; and for comparison chemical analyses were made of the membrane and the extracted materials.

Methods

The osmometer used has been described and figured in a previous paper (1). The apparatus was immersed in a Freas constant temperature water bath and the temperature kept constant to

approximately 0°.1 C. In all experiments reported in this paper the temperature was 25° C. Sodium chloride solutions were used to induce the osmotic movement of water through the membrane. With the peanut membrane (*Arachis hypogaea*), which is rather highly permeable, concentrations of 0.5 M or 0.6 M were found to be suitable; but for squash, cocklebur, etc., which have fairly high resistance to water penetration, strong solutions of sodium chloride were necessary; for use with such membranes a saturated solution was used, that is, saturated at room temperature.

As noted in the previous paper, different membranes of the same species differ in their permeability to water. This difficulty was overcome by using the same membrane under the various conditions of the experiment. In the tables of results each line of a table represents data obtained from a single membrane; it is not possible to compare one membrane with another; the data given by an individual membrane under different conditions must be compared.

Effect of extracting membranes with boiling water

It was noted that heating the seed coat of *Arachis hypogaea* in boiling water increased the rate at which water passed through. Measurements were made to determine the extent of this increase; and other membranes were tested for similar behavior. The method employed was as follows. A membrane was removed from a soaked seed, fitted into the osmometer, and a measurement made of its permeability. It was then removed from the apparatus, placed in cold distilled water, and heated to boiling point and allowed to remain in boiling water for 5 minutes. It was then placed in the osmometer and a reading made of its permeability. This process was then repeated with other membranes. Table I shows the results, which are expressed in milligrams of water passing through a membrane per hour; the same unit is also used in subsequent tables.

The data show that heating the membrane in boiling water increased the permeability of the peanut and almond membranes, but not that of the grapefruit and squash coats. However, the last two membranes are so slightly permeable that a considerable

increase in permeability might escape notice. With more delicate methods an increase under these conditions might appear. In heating the peanut seed coat a brownish extract was obtained, and it is thought that the increase was due in a large measure to the removal of the soluble substances. The nature of these substances will be treated in that portion of the paper dealing with microchemical and chemical tests.

TABLE I

EFFECT OF EXTRACTING MEMBRANES WITH BOILING WATER

Membrane	Area in sq. mm.	Concentration of solution	Rate before extraction	Rate after extraction	Percentage of increase
Peanut	19.635	0.5 M	28.31	66.73	135.7
"	"	"	32.35	82.90	156.3
"	"	0.6 M	35.05	115.59	229.8
"	50.265	0.5 M	89.54	212.31	137.2
"	19.635	0.6 M	54.67	150.00	174.4
"	50.265	0.5 M	34.67	114.30	229.7
"	"	"	88.56	210.00	137.1
"	19.635	0.5 M	40.44	101.10	150.0
Almond	"	Saturated	17.52	105.14	500.1
Grapefruit	"	"	0	0	.....
"	"	"	0	0	.....
Squash	"	"	0	0	.....
"	"	"	0	11.76	.....

TABLE II

EFFECT OF HEATING DRY MEMBRANES

Membrane	Area in sq. mm.	Concentration of solution	Rate before heating	Rate after heating	Percentage of increase
Peanut	19.635	0.5 M	28.31	42.47	50.03
"	50.265	0.8 M	78.86	101.10	28.2

To gain further information on this point, the effect of heating the dry membrane was compared with the effect of heating the membrane in water. The permeability of a membrane was first measured; it was air dried, placed in a glass tube, and the latter heated in water as described. The membrane was then soaked in water, placed in the osmometer, and a reading again made of its permeability. Table II expresses these results.

The results indicated in table II show that part of the increase in permeability after heating in water is due to the heating effect itself, and that not all the increase is due to the extraction of materials from the membrane.

Effect of extracting membranes with hot lipoid solvents

The rôle of lipoid substances present in the membrane in limiting the rate of penetration of water was investigated by determining the permeability of a membrane before and after extraction of the membrane with a lipoid solvent. The solvents used were alcohol, acetone, and ether. After 4 hours' treatment with the solvent, the solvent was removed from the membrane and the latter soaked in water before being placed in the osmometer. Table III shows the results obtained. In this series all membranes were previously heated in boiling water. It will be noted that extracting the

TABLE III

EFFECT OF EXTRACTING MEMBRANES WITH HOT LIPOID SOLVENTS

Membrane	Area in sq. mm.	Concentration of solution	Rate before extraction	Rate after extraction with hot alcohol	Percentage of increase	Rate after extraction with hot acetone	Percentage of increase	Rate after extraction with hot ether	Percentage of increase
Peanut	50.265	0.5 M	92.67	144.45	55.9	.....	.....	169.84	83.3
"	19.635	0.6 M	52.23	92.34	76.8	.....	.....	92.47	77.0
"	50.265	0.5 M	98.40	228.48	132.2	.....	.....	.....	.....
"	"	"	78.86	161.76	112.6	171.87	126.3	.....	.....
"	"	"	151.65	257.80	69.9	.....	.....	279.06	84.1
"	19.635	"	101.10	117.26	15.9	.....	.....	131.43	30.0
"	50.265	"	113.24	242.65	114.3	217.70	92.2	.....	.....
Squash	19.635	Saturated	11.12	99.42	793.9	101.10	809.2	.....	.....
"	"	"	15.51	82.90	434.5	98.07	532.6	.....	.....
"	"	"	11.45	111.22	871.3	119.08	939.8	.....	.....
Almond	50.265	"	60.66	232.55	283.4	234.89	287.2	.....	.....
"	"	"	50.55	279.71	452.3	279.06	452.0	.....	.....
Cocklebur	19.635	"	20.22	48.28	138.8	40.44	200.0	.....	.....
"	"	"	14.83	45.83	209.1	.....	.....	.....	.....
"	"	"	22.24	56.62	154.6	.....	.....	.....	.....
Grapefruit	"	"	0	0	.....	.....	.....	.....	.....
"	"	"	0	10.11	.....	.....	.....	.....	.....
"	"	"	0	0	.....	.....	.....	.....	.....

membrane with lipoid solvents greatly increased the permeability to water. Only in the case of the grapefruit seed did such treatment fail to show an increase. The different solvents did not

show large differences, but in general acetone and ether were more effective than alcohol in increasing the rate, possibly due to their better dissolving power. The effect of the lipoid solvents upon the permeability of the squash membrane is to be noted especially. Most squash membranes will not show any penetration by water under the conditions of these experiments until they are treated with a lipoid solvent. They then become rather permeable to water.

Effect of extracting membranes with cold lipoid solvent

The significance of lipoids in the membranes is well shown by the series of measurements made when the extraction was carried on at room temperature. The unheated membranes were merely placed in bottles of acetone and allowed to undergo extraction at room temperature for 18 hours. The permeability of the membranes was measured before and after such treatment. Table IV shows the results of this experiment.

TABLE IV

EFFECT OF EXTRACTING MEMBRANES WITH COLD LIPOID SOLVENT

Membrane	Area in sq. mm.	Concentration of solution	Rate before extraction	Rate after extraction with cold acetone	Percentage of increase
Peanut	19.635	0.5 M	14.49	60.66	318.6
"	50.265	"	65.71	246.68	275.4
"	19.635	"	36.00	88.29	145.3
"	"	"	24.27	76.84	216.6
Pumpkin	50.265	Saturated	0	251.77	.....
"	"	"	4.04	141.54	.....
Squash	19.635	"	0	118.64	.....
"	"	"	0	107.84	.....
Almond	"	"	17.52	34.37	96.18
"	50.265	"	101.10	153.67	53.21
Grapefruit	19.635	"	0	0	.....

Infiltration experiments

Experiments were made to infiltrate the extracted membrane with the extracted material. Membranes that had been extracted were soaked in the solvent containing the lipoid extract and then exposed to evaporation; by a continuance of this process it was hoped to impregnate the membrane again with the material that

had previously been removed. Table V shows these results. It is apparent that while the permeability is reduced by the infiltration, the decrease does not reach the low point exhibited by the membrane before the original extraction. Apparently the lipoid materials cannot be put back into the membrane in the condition and position in which they existed before extraction. This would indicate an organized distribution of these materials in the membranes in nature.

TABLE V

EFFECT OF INFILTRATING MEMBRANES

Membrane	Area in sq. mm.	Concentration of solution	Rate before extraction	Rate after extraction with hot acetone	Rate after extraction with cold acetone	Rate after infiltration with lipoid extract
Peanut	50.265	0.5 M	65.71	.....	246.68	181.98
"	19.635	"	24.27	.....	76.84	30.33
Pumpkin	"	Saturated	4.08	146.26	.....	84.92
Cocklebur	"	"	16.17	54.59	.....	42.46
Almond	50.265	"	101.10	298.26	.....	232.52
"	"	"	121.34	326.03	.....	258.81

Effect of extracting membranes with calcium chloride

The calcium ion has been shown by HANSTEEN-CRANNER (2) to reduce the rate of water intake by the cell walls of roots, and it was thought desirable to determine whether calcium had similar effects upon the permeability of seed coats to water. Membranes were selected and measurements made of their permeability to water; these membranes were then soaked for 24 hours in calcium chloride solutions of the concentrations described later; the membranes were then rinsed in distilled water, placed in the osmometer, and their permeability again measured. Table VI shows the results. It thus seems that calcium chloride at the concentrations used increased the permeability of the membrane to water. Whether more dilute concentrations would have similar or opposite effects is yet to be determined.

The fact that callose is soluble in calcium chloride solutions would suggest the hypothesis that the increase in permeability is due to the extraction of callose from the membrane. No microchemical differences were noted in membranes before and after

extraction in calcium chloride, however. The peanut seed coat became dark brown in color after treatment in saturated calcium chloride.

TABLE VI

EFFECT OF EXTRACTING MEMBRANES WITH CALCIUM CHLORIDE

Membrane	Area in sq. mm.	Concentration of solution	Rate before extraction	Rate after extraction	Percentage of increase	Concentration of CaCl
Peanut	50.265	0.5 M	89.17	117.96	32.3	Saturated
English walnut	19.035	Saturated	22.92	38.43	67.6	"
Almond	"	"	24.26	48.53	100.0	"
"	"	"	26.96	32.69	21.3	1 gm. in 250 cc. water
Peanut	50.265	0.5 M	205.58	220.06	7.0	1 gm. in 250 cc. water

Microchemical tests

The membranes were examined microchemically to determine the nature of the substances composing them, and to note the effect upon them of the various methods of treatment. Thin cross-sections of the seed coats were made with a freezing microtome. These sections were 12.5–25 μ thick. The substances for which tests were made are given, together with the tests applied in each case. The textbooks of TUNMANN (**6**) and of MOLISCH (**4**) were consulted for directions regarding these tests.

Tests for suberin.—Insoluble in 50 per cent chromic acid and concentrated sulphuric acid; soluble in 3 per cent alcoholic potash after heating; gives the ceric acid reaction; and stains red with Sudan III.

Tests for tannins.—Blue color with dilute ferric chloride, and a violet color with 0.1 per cent gold chloride.

Tests for lipoids.—Soluble in acetone, ether, and hot alcohol; stain red with Sudan III; true fats give crystals when saponified with potassium hydroxide.

Tests for pectic substances.—Stain red with ruthenium red, and dissolve by treatment with 2 per cent hydrochloric acid followed by 2 per cent potassium hydroxide.

Tests for proteins.—Biuret reaction; those containing tyrosine give a red color with Millon's reagent; those containing tryptophane give a violet color by Liebermann's reaction; other protein tests were not found to be suitable because the color of the membranes interfered with the tests.

Tests for cellulose.—Blue color with sulphuric acid and iodine, and dissolves in copper-oxide-ammonia.

Membrane of peanut (*Arachis hypogaea*)

The cells in the layer at *a* (fig. 1) have walls of cellulose and pectin, and their contents consist of granular lipoid substances. The layer at *b* contains coloring matters, of which tannin was shown to be one. A water extract of this seed coat also gives a positive reaction for tannin. The color in this layer is not completely removed either by treatment with hot water or with lipoid solvents. The layer at *c* consists of the old walls of a parenchymatous tissue that, in the mature seed coat, is much compressed. These walls are of cellulose and pectin. It requires a long period of heating in acid and dilute base to cause these cells to fall apart. The membrane at *d* has no visible contents. The outer walls are of cellulose and pectin, but of a type very resistant to chromic acid. When sections of this membrane are treated with chromic acid these walls are the last to be broken down. Furrows extend across the thick wall of this layer, but the remains of protoplasmic connections between the cells could not be detected. Fatty bodies could be seen

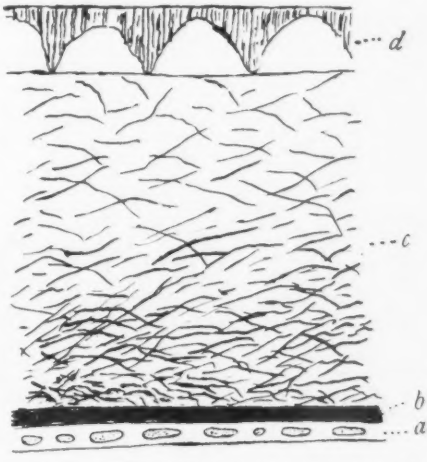

FIG. 1.—Cross-section of seed coat of *Arachis hypogaea*, ×450.

occasionally in the layers *c* and *d*, but saponification crystals were not obtained in place in the tissue.

That lipoid substances are present in the peanut membrane is shown by the analysis of the membrane given later in this paper. It is probable that the lipoids are present in a very fine state of dispersion. No distinctly suberized layers could be detected in the seed coat. All protein tests were likewise negative.

The hot water extraction removes most of the extractable tannins from the membranes, also part of the lipoid materials; the increase in permeability by heating the membrane in boiling water is probably due in large measure to the removal of these substances; and the increase in permeability after extraction with acetone, alcohol, etc., is due to the further removal of lipoid materials. The high permeability of the peanut membrane as compared with other membranes studied is related to its low lipoid content, and especially to the lack of the layers of cells filled with lipoid substances that were found in the other seed coats.

Membrane of cocklebur (*Xanthium pennsylvanicum*)

The walls at *a* (fig. 2) are thick and lignified; those at *b* are much compressed and are composed of cellulose and pectin; at *c* is a suberized layer containing tannins. Tannins also appear in *b*,

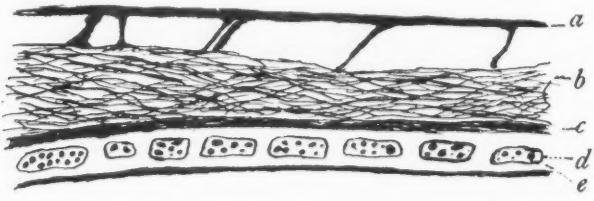

FIG. 2.—Cross-section of seed coat of *Xanthium pennsylvanicum*, ×450

but may have diffused there from *c*. The layer at *d* and *e* is an endosperm layer; the cell walls marked *e* are composed of cellulose and pectin, and the contents *d* are of lipoid substances. Extraction with lipoid solvents does not completely remove the fatty substances from this layer. Protein tests all gave negative results. Layers *a* and *c* are resistant to chromic acid, but layer *d*, *e* breaks up, and the fatty substances flow out in drops.

Membrane of squash (*Cucurbita maxima*)

This is the greenish membrane surrounding the cotyledons, and is obtained from soaked seeds by first removing the horny white spermoderm layer, and then removing the greenish coat from the cotyledons. At *d* (fig. 3) is the greenish layer; at *o* is a compressed layer of the cell walls composed of cellulose and pectin. The inner layer is the endosperm layer, and the cells of this layer are completely filled with fatty substances. The walls are thin and are of cellulose and pectin. In some cases protein reactions were positive in layer *d*, but most tests were negative. No tannins were found and suberized membranes were absent.

FIG. 3.—Cross-section of seed coat of *Cucurbita maxima*, ×450.

It is believed that the high resistence to the flow of water through this membrane is due to the high content of lipoid substances in layer *f*, *m*. These substances are almost entirely removed by treatment with lipoid solvents, and a large increase in permeability results. It is worthy of note that although this membrane is highly resistant to the passage of water through it as compared with other membranes, distinctly suberized layers do not enter into its composition.

Membrane of almond (*Prunus Amygdalus*)

Large cells *f* (fig. 4) were found to extend from the outer surface of the seed coat. These were in some places much distorted in shape and they also did not cover the entire surface. The walls were of cellulose and pectin, and protein tests gave positive results in this layer. At *d* is a thick layer of parenchymatous cell walls giving cellulose and pectin reactions. A suberized layer is found at *e* and tannin reactions were positive in this region. At *m* again is a layer of compressed parenchymatous cell walls. The endosperm layer is at *o*, *s*. The cells are filled with fatty substances (*o*), and the cell walls *s* are composed of cellulose and pectin. These walls are not thick as compared with either the *Citrus* or *Xanthium* coats. The cell contents also are not completely removed by extraction with lipoid solvents.

Membrane of grapefruit (*Citrus grandis*)

Layer *f* (fig. 5) is a mucilaginous mass of cell wall material giving both cellulose and pectin reactions. The slimy character of soaked seeds of this species is due to this layer. At *o* is a thick

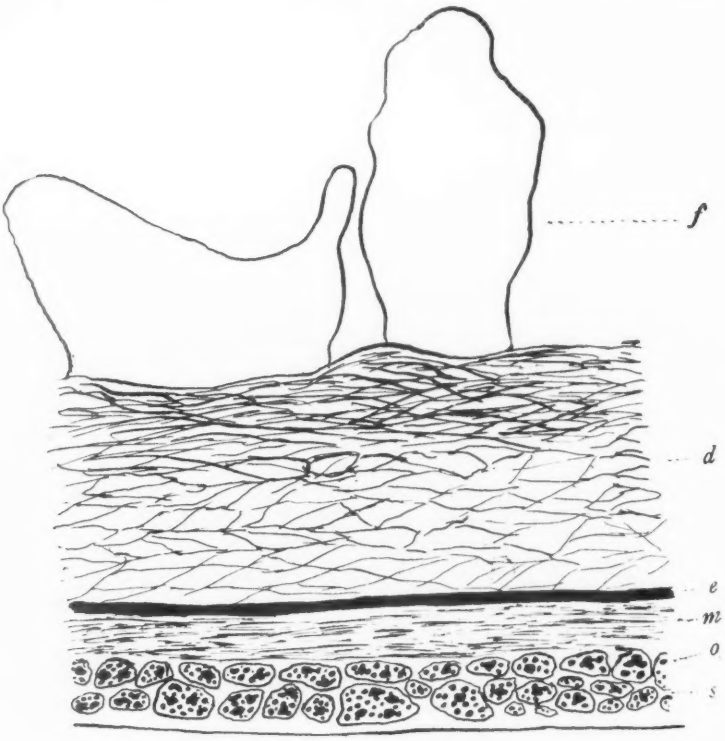

FIG. 4.—Cross-section of seed coat of *Amygdalus communis*, ×450

suberized layer impregnated with tannin and other coloring materials. The inner wall of this layer is especially well suberized. It is more resistant to chromic acid than any layer in any other membranes studied. The thick endosperm layer is marked at *d* and *w*. In some places this layer is 2 cells and in other places only 1 cell thick, the walls are very thick and are composed of cellulose impregnated with pectin. They break apart on treatment with

dilute acids and bases. The cells themselves are filled with fatty substances that are only partly removed by extraction with lipoid solvents. Even after 18 hours of extraction lipoids may still be detected in the coat.

In these various experiments the grapefruit seed coat differed from all others in failing to show increased permeability, or at least a readable rate of water movement after any of the methods of extraction. All tests of this membrane with the osmometer indicated a relatively high resistance of the coat to the passage of water.

A parallel experiment was carried out to give further evidence on this point. This was done by comparing the absorption of

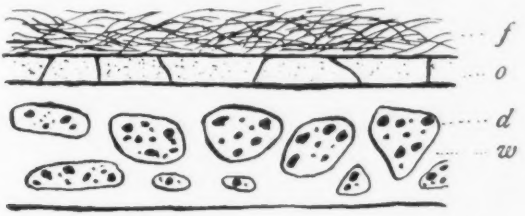

FIG. 5.—Cross-section of seed coat of *Citrus grandis*, ×450

water by the seeds, one lot with coats and the other lot without coats. More than one embryo is usually found in these seeds, so that when the coat is removed the embryos fall apart and accurate weighings are impossible. To overcome this difficulty, a narrow band of seed coat was left around the embryos. This band held the embryos together so that weighings could be made. Table VII shows the results obtained, and curves of these results are shown in fig. 6.

It was found that treating the grapefruit membrane with acids and bases increased its permeability to such an extent that a readable rate of water movement could be obtained. Thus, soaking a membrane in warm 2 per cent sodium hydroxide increased the rate from 0 to 24.26 mg. of water per hour; and in another test soaking the membrane in 2 per cent sodium hydroxide for 24 hours increased the rate from 0 to 72.12 mg. By such treatment, however, the tissue of the seed undergoes a disintegration, so that the

outer yellowish layer *o* (fig. 5) comes off. On using this as a membrane the rate was now found to be 210.27 mg. per hour. This indicates that the inner portion of the membrane is the one that

TABLE VII

RATE OF ABSORPTION OF WATER BY SEEDS OF *Citrus grandis*

Time	Coats on		Coats off	
	Weight in gm.	Percentage of water imbibed	Weight in gm.	Percentage of water imbibed
Commencing	2.423	6.13	2.087	5.67
After 12 hours	2.762	20.9	3.135	58.9
" 36 "	2.888	26.5	3.233	64.3
" 84 "	2.932	28.4	3.171	60.8
" 108 "	2.942	28.9	3.123	54.8
" 144 "	2.986	30.8	3.129	58.7
" 204 "	3.056	33.9	3.139	59.2
Oven dry	2.283	.....	1.971	.....

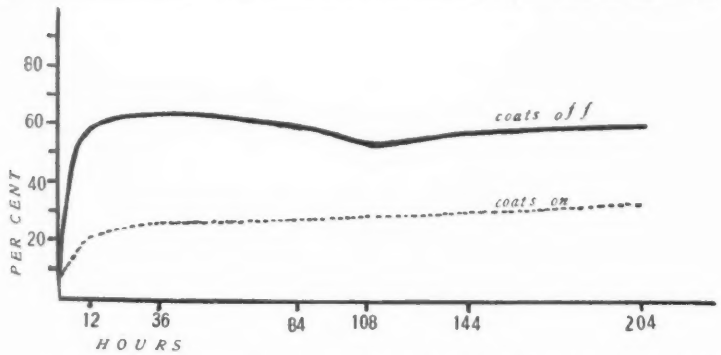

FIG. 6.—Showing rate of imbibition of water by seed of *Citrus grandis;* rate with coats compared with rate without coats; curves obtained by plotting data given in table VIII.

offers the resistance to the passage of water, and it is believed that this resistance is due to the presence of the large amount of fatty substances surrounded by thick pectinized walls.

Results of chemical analysis

An analysis was made of a quantity of the seed coats of *Arachis hypogaea*. A complete analysis for all constituents of the membrane was not attempted, but an examination was made only for

those substances that appeared to be significant as judged by the results of the extraction experiments and of the microchemical tests.

About 10 pounds of unroasted peanuts were shelled, the pods discarded, and the seeds put at once into cold water to soften the seed coats; as soon as the coats were moist enough, they were removed from the embryos, and dried on blotting paper in the air. The water in which the seeds had been soaking was brownish in color; it was made up to a volume of 1000 cc. with distilled water; this was called the "preliminary extract," and later its content of tannins was determined.

The air dried seed coats were divided into two lots, one lot to undergo extraction in a manner comparable to the extractions performed in the experimental work, and the other lot held as a check to determine the original composition of the membrane before extraction. The first lot was extracted with water, by putting the membranes into cold water, raising the temperature of the water to boiling point, and holding it at this temperature for 5 minutes; the water was then drained off through a filter, and the residue washed with cold distilled water until only a faint straw-colored liquid passed through the filter. The filtrate was made up to 1500 cc., and was called the "hot water extract." The residue was vacuum dried, placed in Schleicher and Schüll extraction cups, and extracted in a Soxhlet extractor for 4 hours with acetone. The extract was made up to a volume of 250 ccm. and was called the "acetone extract." The insoluble material was called "residue."

Methods

Tannins were estimated by Proctor's modification of Lowenthal's method (**7**, p. 150). The distribution of tannins in the preliminary extract, in the hot water extract, and in the acetone extract was determined.

The lipoids in the acetone extract were estimated by removing a sample, evaporating in a Jena glass dish, and drying to constant weight in a desiccator. To obtain the amount of lipoid in the hot water extract a sample was taken; strips of filter paper were dipped in the sample until the liquid was absorbed by the filter paper; the

latter was then dried in a vacuum desiccator, placed in extraction shells, and extracted with acetone in a Soxhlet extractor; the weight of the lipoid material was determined by evaporation. The nitrogen content was measured by the Kjeldahl method, and the protein content by the Van Slyke method, both of the latter methods as described by MATHEWS (3). An attempt was made to determine the phosphorus content of the various portions, but it was found to be very low, and insufficient material was left for conclusive analyses. The results of the analyses are summarized in table VIII.

TABLE VIII

RESULTS OF ANALYSIS OF SEED COATS OF *Arachis hypogaea;* DISTRIBUTION OF MATERIALS*

	Preliminary extract	Hot water extract (gm.)	Acetone extract (gm.)	In residue (gm.)	In sample (gm.)
Total solids	.....	3.8940	0.6170	.....	.....
Lipoids	.....	0.2646	0.6170	.....	0.8816
Nitrogen†	.....	0.0507	.....	0.6784	0.7615
Tannins, equivalent to cc. $KMnO_4$	1970.0 cc.	1245.0 cc.	0	.....	.....

* Weight of sample, vacuum dried, 30.1166 gm.

† Amino acid nitrogen not present in determinable amounts.

The following points with reference to table VIII are of interest. The water extract contains two substances that are believed to be of importance in the permeability of the membrane, i.e., tannins and lipoids. Tannins have been reported by REICHARD (5) to be of importance in the permeability of the barley grain to salts, and these results indicate that tannins may also take part in the permeability of the peanut seed coat to water. Remembering the effect of the acetone extractions in increasing the permeability of the membranes, it is interesting to note the low content of lipoid in this extract, only 2.04 per cent of the solid matter. A small amount seems to have great effect in reducing the rate of water movement. From the fact that lipoids were also found in the water extract we may conclude that a part of the increase after the water extraction was due to the removal of lipoids. The lack of proteins in the membrane will also be noted in the table.

Discussion

These experiments point to the important rôle of lipoids in permeability to water. The peanut seed coat, which is the most permeable of the membranes studied, is the one containing the least amount of lipoid substances, and when the lipoids are removed an increase in permeability results. Only in the case of the grapefruit did extraction with lipoid solvents fail to increase the permeability. Reasons for this behavior are suggested.

Suberized layers have usually been considered the main factors in restricting water movement through plant membranes. In these experiments there was no evidence that suberin was a dominant factor. The squash seed coat has no distinctly suberized layers, and yet it is resistant to the passage of water. These statements apply only to the membranes studied; in other membranes, more highly impermeable, suberized layers may be of first importance.

Other substances that appeared to be effective in reducing the permeability of these seed coats were tannins and pectic substances, the latter especially when deposited in thick cell walls.

Summary

1. The rôle of different substances in seed coats in regulating their permeability to water was studied.

2. Membranes were extracted with water, alcohol, acetone, ether, and calcium chloride, and their permeability measured before and after such treatments.

3. Cross-sections of the seed coats were made and tested microchemically to determine the nature of the substances present, and the effect upon them of the different methods of treatment employed in the experiment. A chemical analysis of the seed coat of the peanut and of the extracted materials was made to determine the content of tannins, lipoids, and proteins.

4. Extraction with hot water increased the permeability of the peanut and almond seed coats, the percentage increase ranging from 135 to 500 per cent. Such treatment removed from the peanut membrane the tannins and a part of the lipoid materials.

5. Extraction with hot water did not measurably increase the permeability of the grapefruit and squash seed coats; but these membranes before such treatment were so resistant to the passage of water that an increase could have resulted from the hot water extraction without being detected by the apparatus under the conditions of the experiment.

6. Extraction with hot lipoid solvents increased the permeability of all membranes studied except the seed coat of the grapefruit. The percentage of increase ranged from 15 to 871 per cent.

7. Extraction with acetone at room temperature also increased the permeability of all the seed coats except that of the grapefruit. The percentage increase ranged from 53 to 313 per cent.

8. After a membrane had its lipoid content removed, its permeability was decreased by impregnating it with the lipoid material that had been extracted; but in no case was the permeability reduced to the low point exhibited by it before the process of extraction.

9. Calcium chloride treatments increased the permeability of the membranes, but the cause of this increase could not be determined.

10. The substances found to be factors in determining the permeability of the membranes to water were lipoids, tannins, and pectic substances. Suberized layers were not found to be significant in the membranes studied, and the presence of soluble proteins could not be detected.

I wish to express my thanks to Dr. WILLIAM CROCKER and to Dr. S. H. ECKERSON for helpful advice and suggestions during the progress of this work.

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SEXUALITY OF FILAMENT OF SPIROGYRA[1]

BERT CUNNINGHAM

(WITH PLATES XXIII–XXV)

Is the filament of *Spirogyra* unisexual or bisexual? This has been a question for many years, but the reports of the great majority of modern workers would indicate that they regard the filament as wholly of one sex. The answer to the question hinges upon the presence of zygotes in both of the conjugating filaments. If they occur in only one of the two, the filaments may be said to be unisexual, since one functions as the male and the other functions as the female; if, on the other hand, they occur in both filaments and are formed by scalariform conjugation, the filaments may be said to be bisexual, since the empty cells in each have furnished the male gametes, while the ones in which the zygotes occur previously contained female gametes. The latter case is known as cross-conjugation.

The advocates of the theory of the unisexuality of the filament urge that as a rule all the male gametes arise in one filament and pass over into the other, so that zygotes appear in but one of the two conjugating filaments. They urge also that cases of cross-conjugation are so rare that they should be considered abnormalities and the result of forced conditions. Strangely enough they ignore lateral conjugation in so far as the sexuality of the filament is concerned. On the other hand, those who do not accept this theory have taken two different positions: one, that the gamete is absolutely sexless (HASSALL **17**, p. 130; also PRINGSHEIM, see BENNETT **2**); and the other, that the filament is bisexual, based

[1] This work was done in the Biological Laboratory of Trinity College, Durham, North Carolina, under the direction of Dr. J. J. WOLFE. The writer wishes at this point to thank Dr. WOLFE for his many kind and helpful suggestions. He further desires to acknowledge aid rendered on special questions arising in the course of the work by Professor G. S. WEST, of the University of Birmingham, England, and Professor J. M. COULTER, of the University of Chicago. Thanks are due also to Mr. J. P. BREEDLOVE, librarian of Trinity College, for his great assistance in securing literature on the subject.

principally upon the fact of lateral conjugation. Occasionally a case of cross-conjugation is presented as evidence, but these cases have been so isolated that they have been of little value. A clear case, then, of cross-conjugation occurring normally in a species would certainly strengthen the position of the advocates of the bisexuality of the filament.

VAUCHER (**28**), who was apparently the first to work upon *Spirogyra*, at least upon the problem of its reproduction, figures it in most cases in cross-conjugation. Figs. 1–4 of pl. XXIII are photographic copies from his plates. In his general description of the *Conjuguee* he says "ordinarily one of the filaments gives while the other receives throughout its entire length. Still it is not rare for the same filament to give in one part of its length and receive in another in such a manner that some of the cells of the tube are filled and some are emptied." Again, "while a tube usually gives or receives throughout its entire length, still it frequently happens, as I have said before, that some give and receive almost alternately." In regard to *Conjuguee condensee*, he says "the berries are indifferently lodged in either tube" (VAUCHER **28,** p. 69).[2] Such statements seem to point clearly to cross-conjugation, but when we remember that VAUCHER, probably because he was unacquainted with the phenomenon, did not figure or describe lateral conjugation, and that he was dealing in part with species which normally reproduce in this manner, we have reason to believe that he was really observing a combination of lateral and scalariform conjugation which might easily have been mistaken for cross-conjugation. Just such conditions have been observed by the writer. Pl. XXIII, fig. 9, shows diagrammatically such a case that came under his observation. At first glance the position of the zygotes in the 2 filaments gives the appearance of cross-conjugation. Upon closer investigation it is found that the contents of cell (*a*) probably passed into (*b*), (*c*) and (*d*) represent another lateral conjugation, (*e*) and (*f*) have conjugated in the regular scalariform manner with (*g*) and (*h*), (*i*) and (*j*) are lateral, and (*k*) entirely failed to conjugate.

[2] Free translations.

VAUCHER has figured 6 species of *Spirogyra*, 2 of which are shown in scalariform conjugation and 4 in cross-conjugation. I have arranged these species in table I so that the methods of reproduction assigned by the different writers may be compared.

TABLE I

SPECIES ACCORDING TO VAUCHER	METHOD OF REPRODUCTION		
	Vaucher	Wolle	DeToni
Allongee	Scalariform	Scalariform	Lateral
Adherente	Scalariform	Scalariform	Not stated
Majeure	Cross	Scalariform	Not stated
Portiquis	Cross	Scalariform and aplanospores	Not stated
Condensee	Cross	Scalariform	Often lateral
Renflee	Cross	Scalariform and lateral	Often lateral

At first glance there does not seem to be much uniformity, but if we assume that where DETONI does not state the particular method of conjugation that the scalariform method is meant, we find that WOLLE and DETONI agree that 5 of these species have scalariform conjugation. They also agree in assigning lateral conjugation to *renflee*. However, DETONI alone states that *condensee* conjugates laterally, while WOLLE alone states that *portiquis* forms aplanospores. In these 3 cases, therefore, it would be possible to have combinations with the appearance of cross-conjugation. *Majeure*, however, according to both WOLLE and DETONI, conjugates only in the usual scalariform manner. Figuring this species in cross-conjugation by VAUCHER is rather surprising, as will appear in the discussion which follows.

Since there have been but few cases of cross-conjugation reported, we have reason to doubt its occurrence in as many species as figured by VAUCHER. Furthermore, no other observer up to the present time has recorded its occurrence in any of these species. However, the species discussed in the present paper has been tentatively identified as *S. inflata* (Vauch.) Rahb., which according to RAHBENHORST would include *Conjuguee renflee* of VAUCHER.

Recognizing, then, the facts that (1) VAUCHER was unacquainted with lateral conjugation, (2) a combination of lateral

and scalariform conjugation resembles true cross-conjugation in appearance, (3) he has figured cross-conjugation in 3 species in which this appearance would naturally occur, due to this combination, (4) no other observer has reported any of these species in cross-conjugation, the writer feels that there is good ground to doubt the observation of true cross-conjugation by VAUCHER.

HASSALL (**17**) also has figured cross-conjugation, but does not describe it for any of the species thus figured, although he does mention it in the general description of the Zygnemaceae. Figs. 6 and 8 of pl. XXIII are reproductions from his plates showing this phenomenon. While HASSALL figures and describes cross-conjugation, he nowhere claims to have observed its occurrence. One of the contemporaries of HASSALL, in reviewing his book (HASSALL **18**), says "it is unfortunate that the author has not pointed out the cases in which the figures are not the result of his own observations but copied from published plates." Certain cases are cited in which HASSALL'S plates were taken from published plates, and these tend to cast some doubt on the source of his plates on cross-conjugation, although they are not among those cited. The writer has been seeking the originals of these borrowed plates, but as yet has not been able to locate them, and is therefore uncertain as to whether or not they exist. BENNETT (**2,** p. 432), in reviewing the general field, says in regard to HASSALL "it is quite possible that the statement may be the result of an error of observation; I have often been deceived in this way." Further, HASSALL claims to have discovered lateral conjugation, and with this as his basis he lays great stress upon the act of conjugation as being without sex, explaining the movement of the gamete by the "law of universal gravitation" (HASSALL **17,** p. 132). He gives the following reasons for his belief: (1) both cells are alike; (2) reproductive bodies are surrounded by the heavy wall solely for protection; (3) spores arise in the same species, both with and without conjugation; and (4) there is conjugation but "no mixing of the endochrome." As a conclusion he says "thus, so far as can be presumed, the information already acquired would be opposed to the belief in the existence of sex as applied to the cells of *Conferva*."[3]

[3] This genus as used by HASSALL included *Spirogyra*.

With this as a basis one is not surprised to find him ascribing cross-conjugation as a character of the genus.

BENNETT (**2**) states that CLEVE (**8**) has figured 2 cases which may be called cross-conjugation. The writer, however, thus far has been unable to secure this monograph.

BESSEY (**4**) figures a case which he calls cross-conjugation. This figure is reproduced here (pl. XXIII, fig. 7). It is to be noted that in his description no mention is made of cross-conjugation, although it is made later (BESSEY **5**). If the figure is complete, it is by no means a conclusive case, as it shows only one pair of conjugating cells. The presence of a zygote in filament *A* can be explained as the formation of an aplanospore after the cell had put forth the conjugating tube. WEST (**29,** fig. 64) shows a case of false cross-conjugation occurring in this manner. The idea is further supported by the observation of the writer, and diagrammed on pl. XXIII, fig. 9. Again, the cell in the filament *A*, if in cross with *B*, should bear an oval zygote, since the oval form seems to be dominant to the spherical. This fact was observed by BESSEY, as he states that the zygote of *S. protecta* is oval in shape, but those of *S. majuscula* are spherical, and that the hybrid between these two assumes the oval shape characteristic of *S. protecta*. He does not apply this, however, to the zygote formed in filament *A*, which is spherical. If the cell referred to is in cross with another filament, that filament should be shown. The figuring of the canal is not sufficient evidence, as has been shown. Furthermore, this hybrid is undoubtedly a forced condition. The strenuous efforts of *S. majuscula* to reproduce are shown in another case cited by BESSEY (**6**), in which this species tries to hybridize with *Mesocarpus*.

BENNETT and MURRAY (**3,** p. 266) say "as DEBARY (**11**) has pointed out, ... one of the two filaments is entirely emptied, while the other is completely filled with zygospores." To this they have added a footnote "HASSALL, however, figures and asserts to the contrary."

WEST (**30,** p. 125) refers to the footnote of BENNETT and MURRAY, but maintains that the phenomenon is rare. He states that he has seen but a single case, and that was in *S. gracilis* (WEST **29,** p. 47). G. S. WEST, in a more recent personal com-

munication, says that he regards the phenomenon as rare, not having seen more than half a dozen cases of it. However, he sees no reason why it should not occur, as it represents much the same phenomenon from the sex standpoint as lateral conjugation. In both cases there must be a differentiation of sex in the one filament. A very distinct difference, however, will be pointed out later.

COULTER (**10**, p. 40) briefly describes cross-conjugation but does not assign it to any species.

On the other hand, the great majority of botanists doubt the occurrence of cross-conjugation, and with it the bisexuality of the *Spirogyra* filament. AGARDH (**1**), according to HASSALL (**15**), states that one filament is always giving and the other always receiving. WOOD (**32**) mentions scalariform and lateral conjugation, but not cross-conjugation. COOKE (**9**) has 11 plates of *Spirogyra*, but does not figure cross-conjugation. DEBARY (**11**) states that one filament gives and the other receives. WOLLE (**31**) figures and describes 39 species of *Spirogyra*, citing VAUCHER and HASSALL, but does not mention cross-conjugation. HABERLANDT (**14**), according to KLEBS, holds that the filaments are distinctly sexual. In order to verify this statement, KLEBS (**22**) grew *Spirogyra* on nutrient agar, but found that a filament would not conjugate with itself, and therefore concluded that it was all of one sex. This experiment might prove the case for one species, but it hardly appears just to use it as the basis for a sweeping statement that bisexuality does not occur in *Spirogyra*. MOTTIER (**24**) cites this experiment as a basis for belief in the unisexuality of the filament. DETONI (**12**) absolutely ignores cross-conjugation, although he cites the plates of both VAUCHER and HASSALL in his descriptions of the species figured in this condition by them. LOTSY (**23**) states that there is a distinct difference between the male and the female filaments. OLTMANNS (**25**, p. 64) states specifically that we have to do with male and female filaments. HERTWIG (**19**) makes a similar statement. ENGLER and PRANTL (**13**) speak of the visible difference between the male and female filaments. ROBERTSON (**26**), who grew *Spirogyra* extensively under abnormal conditions, did not find a case either of cross or lateral conjugation. Since cross-conjugation did not occur in his

own experiments and is so exceedingly rare in the work of others, he thinks it must be considered a very unusual abnormality. YORK (**33**), who worked several years on the sexuality of *Spirogyra*, seeking methods for determining the sex before conjugation, says "zygotes were never found in both filaments, but only in the one containing the greater amount of food. The male and female are morphologically and physiologically different."

Summarizing, it appears that the evidence for bisexuality is based (1) upon work done over 100 years ago, when the importance of cross-conjugation was not realized, and has not been verified since; (2) upon lateral conjugation, a strong basis ignored by the unisexualists; (3) upon the chance observations of CLEVE, BESSEY, and WEST. At this point it is interesting to note that HASSALL figures no species in cross-conjugation that was thus figured by VAUCHER; that BESSEY's species is not that of either HASSALL or VAUCHER; and that the one cited by WEST is still different. Thus these would all appear to be abnormalities.

On the other hand, the advocates of unisexuality urge (1) that KLEBS found that a filament would not conjugate with itself, hence it is of one sex; (2) that the work of VAUCHER needs verification; (3) that the figures of HASSALL may have been taken from older works; (4) that, since the species figured by VAUCHER and HASSALL are common, the phenomenon should have been observed by modern investigators; (5) that specialists have seen but few cases, not more than a dozen, and these have been called abnormalities because of their rareness; (6) that experimentalists who have spent years on the sexuality and abnormal conjugation of *Spirogyra* have not observed cross-conjugation. All these things point to the unisexuality of the filament.

If, however, as stated in the beginning of this paper, a true case of cross-conjugation of *Spirogyra* should occur normally to any extent, it would settle the question, for one species at least. A species in this condition was found by the author while making a collection of algae along a stream near Durham, North Carolina, on April 1, 1915. The water stood in pools on the low ground, and it was from one of these pools that the collection was made. There was comparatively little of this species mixed with a larger *Spiro-*

gyra and some germinating *Vaucheria*. The phenomenon of cross-conjugation was not observed until the material had been brought into the laboratory, and heavy freshets prevented further collection. However, from this interwoven mass, not larger than a pea, more than 70 slides have been prepared showing cross-conjugation. Some of the slides have several distinct pairs of filaments in this condition. Considerable effort was made to secure long filaments, but this was unsuccessful on account of the intricate tangling of the mass. This species was again collected early in April 1916, in approximately the same locality, showing essentially the same phenomena as the earlier collection, but, owing probably to deficient rainfall, was not abundant and hence it was not possible to add any important facts not shown by the material gathered the year before.

A careful investigation of the material shows that all the known forms of reproduction in *Spirogyra* are represented in this species. While aplanospores occur, they are not found frequently, and they are hard to identify. The regular zygotes are formed by 3 distinct methods. The most common is the well known scalariform method, in which the 2 conjugating filaments have the appearance of a ladder, and the gametes travel in only one direction, so that one filament contains all the zygotes. This has been followed for 20 to 25 pairs of conjugating cells. Zygotes are formed also by lateral conjugation, the contents of one cell passing into the adjoining cell of the same filament. This is accomplished in this species by the bulging of the cell wall away from the septum at one side until there is a small opening left between the 2 cells through which the contents pass. In general appearance the zygotes are like those formed by scalariform conjugation. Usually the cells follow the law laid down by HASSALL (**16,** p. 34) that 2 males alternate with 2 females. This applies only to those filaments in which lateral conjugation occurs alone. In this species it is frequently accompanied by genuflexions. This method (lateral conjugation) occurs in filaments that are also conjugating in the usual scalariform manner. Zygotes are further formed by true cross-conjugation in which "there is the formation of a perfectly normal zygospore in each of the conjugating filaments." Here,

again, the zygotes have the appearance of those formed by scalariform conjugation. This has been followed for 16 pairs of conjugating cells. In this case they are all in conjugation, and, strangely enough, there are 8 zygotes in each filament. These filaments are shown schematically in fig. 1 of pl. XXV, and in part in fig. 1 of pl. XXIV. This is the only pair of filaments with any considerable number of zygotes thus far found which shows the same number in each of the conjugating filaments. A glance at the plates will emphasize the fact that, in general, there is no such regular order. Whole filaments would probably shed further light upon the occurrence. Moreover, filaments are found frequently in which both true cross-conjugation and lateral conjugation occur.

Much care is needed in the study of cross-conjugation, as there are many chances for error. As previously stated, there are combinations of scalariform and lateral conjugation that at first appear to be cases of true cross-conjugation. The writer has used the utmost care and has been compelled to discard a number of slides that at first were thought to show cross-conjugation. Only such cases as have complied with the following rules have been regarded as in cross-conjugation: (1) zygotes must occur in both filaments, the swelling of the egg cell is insufficient evidence; (2) the connecting tube must be visible; (3) the male cell must be empty; (4) end cells must be discarded unless the preceding conditions are met, since lateral conjugation may have occurred.

Slides have been permanently mounted in glycerine and from these the microphotographs of pl. XXIV were taken. They are made at 225 diameters. The plate shows the original photographs, and no "retouching" has been done either on the plates or the prints. Fig. 1 was made from a plain glycerine mount; while the others were from slides stained either with iodine or Magdala red in order that the cell walls might be made a little clearer. Figs. 2 and 6 show several filaments conjugating with each other. In the other figures only 2 filaments are involved. These are, however, very clear cases.

Schematic drawings have also been made (pl. XXV). In these the writer has laid the filaments parallel on the paper, regardless

of their twisting and turning on the slide. In each case, however, he has retained all the cells, whether conjugating or not, from the first to the last pair of conjugating cells in the filaments. When 3 or more filaments were found conjugating, they have been laid parallel also, and as nearly as possible in their relative position. The writer has adopted the schematic method in this paper in order that the reader may grasp more easily the relative position of the empty cells, unchanged cells, and the zygotes. Diagrammatic drawings can be followed more readily than the windings of the camera drawing, as may be seen by a comparison of fig. 1, pl. XXIV, with fig. 1, pl. XXV. The former is a microphotograph of a portion of the pair of conjugating filaments schematically represented in the latter. Furthermore, the diagrammatic method consumes considerably less space.

Two of these slides were exhibited at the meeting of the North Carolina Academy of Science in May 1915, where they were observed by a number of botanists. Other slides were sent to Professor G. S. WEST, who has referred it to *S. inflata*.

The species of *Spirogyra* under discussion is single banded, with 2 or 3 turns of the band, and has the cell membrane replicate at the ends. The length of the vegetative cell is about 80 μ, and the width about 15 μ. The zygote cell is swollen on both sides. The zygote length is about 43 μ and the width about 28 μ; zygote oval, considerably pointed, brown at maturity. In these characters it follows closely the descriptions for *Spirogyra inflata*. On the other hand, it is to be noted that the connecting tubes are always put out by the male cell and fuse directly with the cell wall of the female cell. This is true regardless of which filament furnishes the male gamete, and would indicate that the sex character was present even before the conjugation tube was put forth.

This species differs also from *S. inflata* in the phenomenon of cross-conjugation, which has not been ascribed to it by any modern work available to the writer. LOTSY (**23**, p. 198) states that there is a difference between the male and female filaments of *S. inflata*, which can signify scalariform conjugation only. WOLLE (**31**) asserts that conjugation may be either lateral or scalariform. DETONI (**12**, p. 766) gives *Conjuguee renflee* of VAUCHER (citing VAUCHER,

pl. V, fig. 3) as synonymous with *S. inflata*, and, although VAUCHER figures it in cross-conjugation (pl. I, fig. 4), DETONI merely states that conjugation is often lateral. Since *S. inflata* has been under observation for so long, and these conditions have not been recognized as characters, it would seem that we must either form a new species for this plant or include these conditions in the description of *S. inflata*. The writer is opposed to the multiplication of species, but these are such distinct characteristics that plants showing them should, it would seem, be classed separately. Final decision in this matter, however, must be reserved until the writer or someone else has had opportunity for further investigation.

The occurrence of this species presents some new problems in the general theory of sex as it applies to the filament of *Spirogyra*. The work on the cytology of this genus has not been entirely satisfactory, owing to the difficulty of staining and counting the chromosomes. CHMIELEWSKI (**7**), in 1890, saw evidences of reduction but was unable to count the chromosomes (JOHNSON **20**). In 1899 KLEBAHN (**21**) followed the reduction in the desmids, and 12 years later TRÖNDLE (**27**) succeeded in counting the chromosomes in *Spirogyra* and found that a reduction takes place in the germination of the zygotes. He further found that 4 nuclei were formed, 3 of which degenerate, while "the fourth remains as the nucleus of the single embryonic plant." Evidently the sex factors are separated, and one or the other of them is thrown out in this reduction, since a filament wholly of one sex results.

In the case of lateral conjugation, however, it would seem that reduction cannot take place in the zygote, as both sexes are present in the filament. Moreover, it would seem that reduction takes place in the divisions just preceding reproduction. This may have occurred in the last division before conjugation. Let [♂ ♀] represent a cell which upon division separates the male and female factors [♂ | ♀]. These conjugating would give alternate zygotes and empty cells. In this case conjugation is assumed to take place between gametes derived from the same mother cell. If, however, they should so divide throughout the filament that the male of one mother cell should adjoin the female from another, we should have the alternation of male and female, so that con-

jugation might take place between daughter cells of different mother cells [♂|♀|♂|♀]. But if they should so divide that the female of one mother cell adjoins the female of another, we should have this occurrence [♂|♀|♀|♂], which is very characteristic of lateral conjugation. HASSALL (**16**), observing such a phenomenon, stated that in lateral conjugation there were always 2 empty cells separated by 2 zygotes. WEST (**29,** pl. V, figs. 72, 73) also figures this condition. WOLLE (**31**) makes a similar statement which, however, is not borne out by his plates, since in several cases he has described as lateral conjugation conditions which according to his own figures are manifestly aplanospore formations (WOLLE **31,** pl. 132, figs. 5, 8; pl. 133, fig. 1; pl. 134, fig. 1).

This characteristic appearance may be brought about, however, by another method of division. If the cells adjoining should so divide that the male and female elements alternate [♂|♀|♂|♀], and if this were followed by the subsequent division of all the cells, a filament would be produced which upon conjugation would contain 2 zygotes alternating with 2 empty cells [♂|♂|♀|♀|♂|♂|♀|♀]. If, however, the division should so occur that male adjoined male and female adjoined female [♂|♀|♀|♂], then a further division would produce a filament containing 4 consecutive males and 4 consecutive females [♂|♂|♀|♀|♀|♀|♂|♂].

Assuming that reduction has been retarded until just previous to reproduction in lateral conjugation, it would be possible for us to have an alternation of empty cell and zygote or an alternation of 2 empty cells and 2 zygotes. If these filaments should cross-conjugate (and there is no reason why they should not), they might produce results such as diagrammed in pl. XXV, figs. 9, 13; or pl. XXIV, fig. 3. The greatest number of consecutive zygotes would be 2. Assuming further that the second division has taken place, producing 4 nuclei, as usually occurs in the formation of sex cells, the greatest number of consecutive zygotes would be 4.

These numbers cannot explain the conditions figured on pl. XXV, figs. 1, 2, 3, or those of pl. XXIV, figs. 1 and 4, and many others

which the writer has observed but not diagrammed, since cases in which there are more than 4 consecutive zygotes or empty cells show that division has occurred once or more after reduction. Here, then, is a distinct and characteristic difference between lateral and cross-conjugation. In lateral conjugation there can be no further cell division after reduction is complete, but in cross-conjugating filaments there may be. A study of the plates shows that in the case of fig. 2, pl. XXV, division must have occurred 3 times subsequent to reduction in part of the cells at least, in order to produce the 11 consecutive males there shown.

From the foregoing it would seem that the phenomenon of cross-conjugation lies between lateral and scalariform and partakes of some of the characters of each. Like the former, the reduction does not occur in the zygote, but is retarded, and none of the potential gametes is lost. Like the latter, division continues after reduction has taken place. For these reasons it would seem that the filament of *Spirogyra*, in this species and in those with lateral conjugation at least, must be homologized with the sporophyte of higher plants. With these facts as a basis, the following conclusions seem to be justified:

1. Bisexuality of the filament does occur in certain species of *Spirogyra*, but not necessarily in all species.

2. Reduction may occur in the zygote, in which case a filament wholly of one sex arises, or reduction may occur just previous to reproduction, in which case none of the nuclei degenerates, and filaments of a bisexual nature are produced, which would conjugate either laterally or by cross-conjugation.

3. Cell division may take place subsequent to reduction, some cases showing 3 divisions, and this is an essential difference between lateral and cross-conjugation, since the latter may continue cell division after reduction is complete but the former apparently does not.

4. The filament of *Spirogyra*, in this species and those with lateral conjugation, is homologous with the sporophyte of higher plants.

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EXPLANATION OF PLATES XXIII–XXV

PLATE XXIII

FIG. 1.—*Conjuguee majeure* (princeps), in cross-conjugation; copy VAUCHER, pl. IV, fig. 3; *Spirogyra nitida*.

FIG. 2.—*Conjuguee portiquis* VAUCHER, in cross-conjugation; copy VAUCHER, pl. V, fig. 1; *Spirogyra porticalis* (Meull.) Cleve.

FIG. 3.—*Conjuguee condensee* VAUCHER, in cross-conjugation; copy VAUCHER, pl. V, fig. 2; *Spirogyra condensata* (Vaucher) Kütz.

FIG. 4.—*Conjuguee renflee* VAUCHER, in cross-conjugation; copy VAUCHER, pl. V, fig. 3; *Spirogyra inflata* (Vaucher) Rabenh.

FIG. 5.—*Spirogyra gracilis* in cross-conjugation; copy West (**29**) pl. V, fig. 81.

FIG. 6.—*Zygnema intermedium* HASSALL, in cross-conjugation; copy HASSALL (**17**) pl. 38, fig. 8; *Spirogyra Weberi* Kütz.

FIG. 7.—*Spirogyra protecta* and *S. majuscula* hybridizing; claimed to be in cross-conjugation; copy of BESSEY (**4**).

FIG. 8.—*Zygnema orbiculare* HASSALL, figured in cross-conjugation; copy HASSALL (**17**) pl. 19, figs. 1, 2; *Spirogyra maxima* (Hass.) Wittr.

FIG. 9.—Schematic drawing of "false cross-conjugation" as observed by the writer.

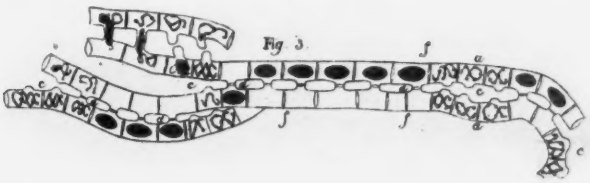
PLATE XXIV

FIG. 1.—Microphotograph of material mounted in glycerine and unstained; ×225.

FIGS. 2–7.—Microphotographs of material mounted in glycerine, and stained with iodine and Magdala red; ×225.

PLATE XXV

FIGS. 1–19.—Schematic drawings of cases of cross-conjugation observed by the writer.



1

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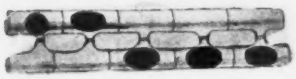
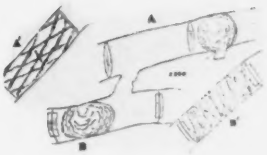
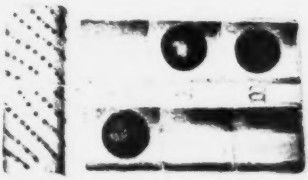
5



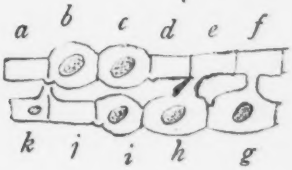
6



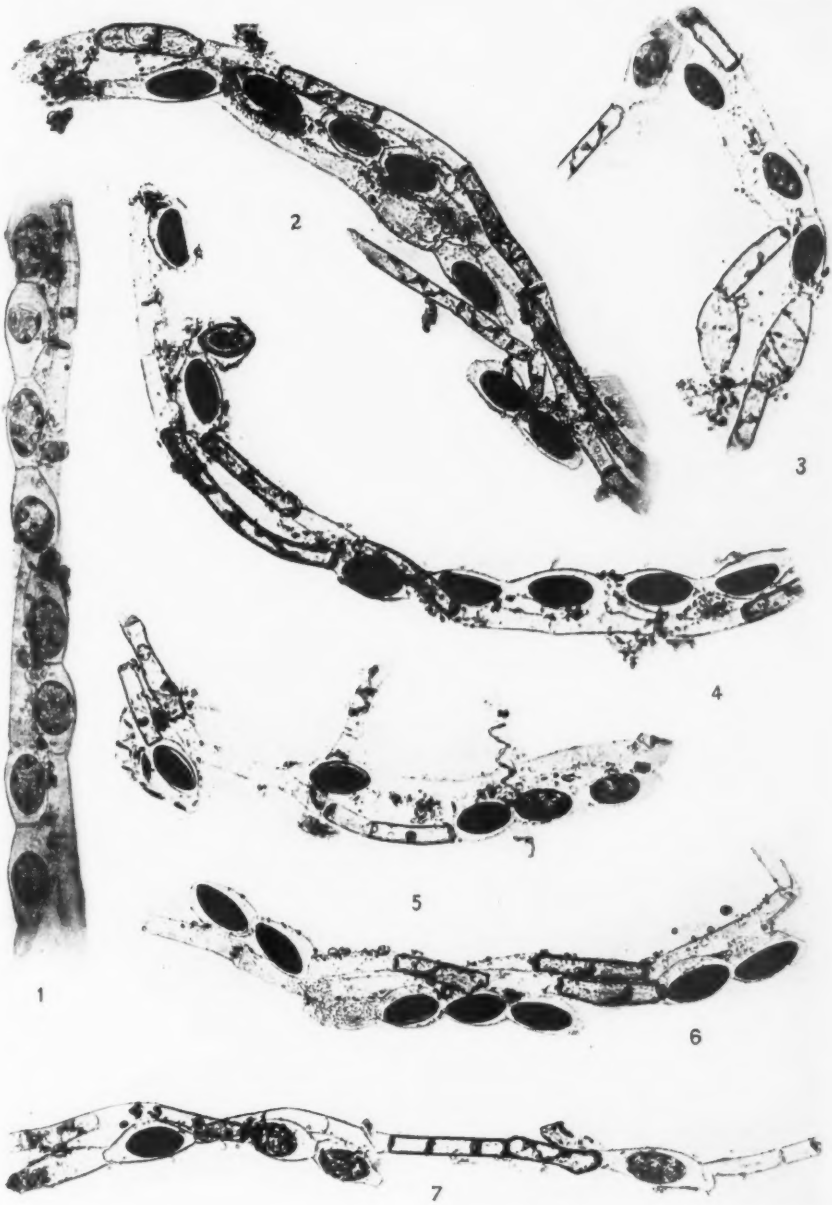
7



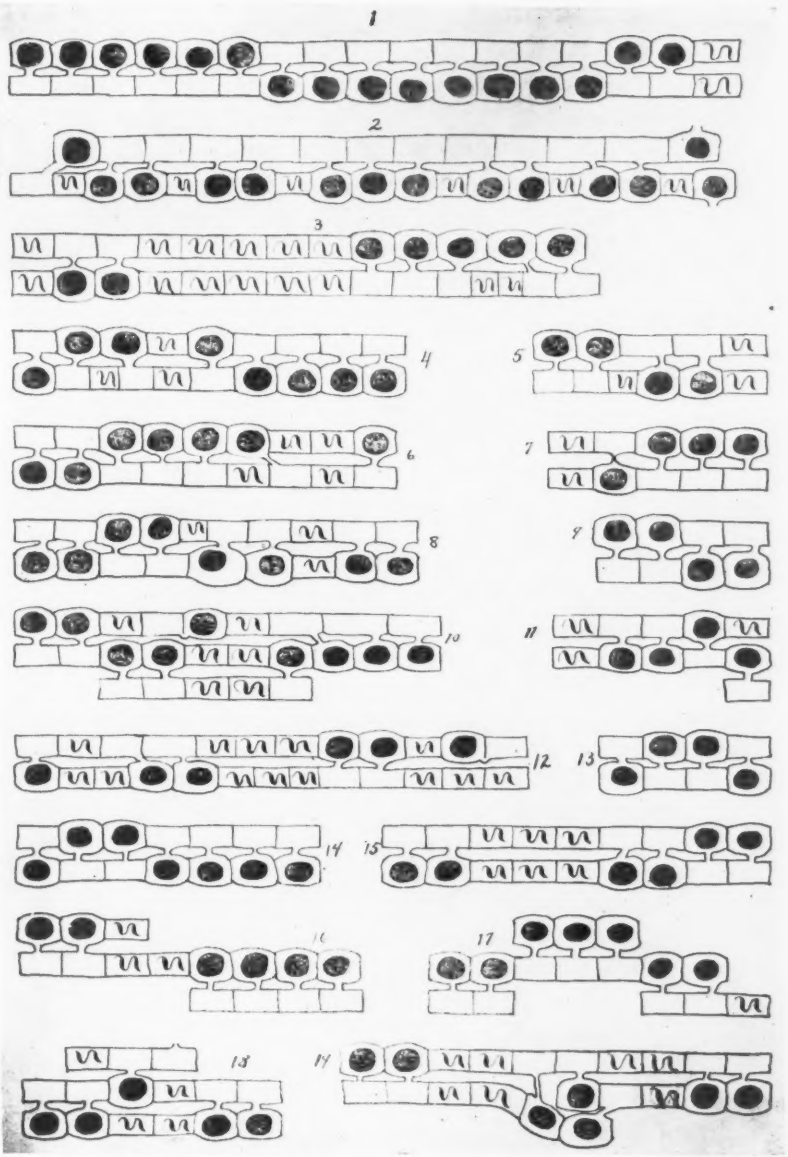
8



9



CUNNINGHAM on SPIROGYRA



CUNNINGHAM on SPIROGYRA

ORANGE RUSTS OF RUBUS

J. C. ARTHUR

(WITH ONE FIGURE)

Much interest has been taken recently in the short cycle rusts, especially since the startling discovery by KUNKEL,[1] 4 years ago, that a rust, indistinguishable from the aecia of *Gymnoconia interstitialis*, would produce promycelia. The year following, FROMME[2] and the writer demonstrated the telial nature of *Aecidium tuberculatum* Ellis and Kellerm., previously treated as a probable heteroecious rust. KUNKEL's discovery stimulated researches by OLIVE and WHETZEL[3] upon the short cycle rusts of Porto Rico, leading to the detection of 5 aecidioid forms previously placed under the form genus *Aecidium*, and of one uredinoid form quite unlike anything heretofore known.

KUNKEL followed up his discovery with supplementary studies, which he has embodied in a paper[4] dealing with the facts, so far as known, pertaining to the blackberry orange rust and of their probable bearing upon questions of relationship and evolution. He has concluded that there are in the United States two independent but in part similar rusts on *Rubus*, one a long cycle form, which he identifies with the *Gymnoconia interstitialis* of Europe, and the other a short cycle form, for which he uses the name *Caeoma nitens*, first given by SCHWEINITZ to a collection made in North Carolina.

No clear morphological characters were found by which to distinguish the short cycle form from the aecia of the long cycle form, although in germination the two behave quite unlike. It is assumed

[1] KUNKEL, L. O., The production of a promycelium by the aecidiospores of *Caeoma nitens*. Bull. Torr. Bot. Club **40**:361. 1913; see also Amer. Jour. Bot. **1**:37. 1914.

[2] FROMME, F. D. and ARTHUR, J. C., A new North American *Endophyllum*. Bull. Torr. Bot. Club **42**:55. 1915.

[3] OLIVE, E. W. and WHETZEL, H. H., *Endophyllum*-like rusts of Porto Rico. Amer. Jour. Bot. **4**:44. 1917.

[4] KUNKEL, L. O., Further studies of the orange rusts of *Rubus* in the United States. Bull. Torr. Bot. Club **43**:559. 1916.

by KUNKEL that whatever the origin or degree of relationship of these two forms, they are to be considered and treated as distinct, a view in which the writer fully concurs.

A considerable difference in the distribution of the two forms is evident from KUNKEL's studies, the long cycle form being more common northward and the short cycle form more common southward. So far the latter is not known in the Old World. Moreover, it is the short cycle form that appears to possess the chief economic importance in America by attacking cultivated varieties of *Rubus*, especially blackberries.

From these considerations it is evident that the two forms as a matter of convenience should be designated by different names, whatever views may be held as to their relationship. Since the appearance of KUNKEL's first paper, appeals have been made to the writer a number of times for the correct application of the several names which have been in use. To meet an evident need, therefore, I have concluded to present the following arrangement of nomenclature and distribution, based upon such facts and material as are at hand.

The generic name *Gymnoconia* was founded by LAGERHEIM[5] in 1894 to more clearly recognize taxonomically the culture work of TRANZSCHEL[6] at Petrograd, Russia, performed during the summer of 1892, by which he genetically connected the caeomoid aecia of *Rubus saxatilis* with the puccinioid telia (*Puccinia Peckiana* Howe) on the same host. The same connection was made two years later by CLINTON[7] at Urbana, Illinois, using "*Rubus villosus*," the native blackberry of the region, being without doubt what is now called *R. nigrobaccus*. The genus *Gymnoconia*, therefore, represents the long cycle form, for which *interstitialis* is the oldest specific name. This was given to the aecia on *Rubus arcticus* from Kamchatka in 1820 by SCHLECHTENDAHL.[8] There can be no doubt,

[5] LAGERHEIM, G., Tromsö Mus. Aarsh. **16**:140. 1894.

[6] TRANZSCHEL, W., Culturversuche mit *Caeoma interstitiale* Schlechtd. (*C. nitens* Schw.). Hedwigia **3**:257. 1893.

[7] CLINTON, G. P., Relationship of *Caeoma nitens* and *Puccinia Peckiana*. BOT. GAZ. **20**:116. 1895.

[8] SCHLECHTENDAHL, D. F. L., Horae Phys. Berol. 96. 1820.

therefore, regarding the correct name for the long cycle form, unless exception be taken to the use of a specific name founded on the aecia, in which case the designation would become *G. Peckiana* (Howe) Trotter.

The short cycle form was first named by SCHWEINITZ[9] in 1822, as *Aecidium nitens*, in a paper on the fungi of North Carolina, the host being reported as *Rubus strigosus*, but a recent study of the type collection shows it to be in all probability *R. Enslenii* Tratt. This region is far south of the known range of the long cycle form, which may be considered sufficient evidence that SCHWEINITZ had only the short cycle form in hand. The specific name has been variously combined, usually in an unfortunate way, but in no case with a distinctive generic name.

Many short cycle genera have now been established. Those which come nearest to the desired characters for the *Rubus* rust are the aecidioid genus *Endophyllum*, and the uredinoid genus *Botryorhiza*. What is now needed is a caeomoid genus, for which no name exists. If any one feels reluctance in placing the telia of a short cycle form in a different genus from the aecia of a long cycle form from which they cannot be distinguished morphologically, let him reflect that he does not hesitate to call the common salt-grass rust *Uromyces Peckianus* Farl., when it has numerous mesospores, and *Puccinia subnitens* Diet., when the mesospores are few, a closer relationship than in the blackberry rusts. Other such accepted anomalies, in the application of the genera *Uromyces* and *Puccinia*, could be cited. After all, we are not near enough to a nomenclature of the rusts along well considered genetic lines to debar us from using such names as enable one to form the clearest concepts and to convey thoughts in the least ambiguous manner. To meet these requirements short cycle forms require separate generic designation from long cycle forms.

In proposing a generic name for the short cycle orange rust of blackberries and raspberries, I take the opportunity to recognize the distinguished service which Dr. LOUIS OTTO KUNKEL has rendered to uredinology, not alone by the discovery of the true nature of this rust through the use of free surface germination of the

[9] SCHWEINITZ, L. D., Schrift. Nat. Gesell. Leipzig 1:69. 1822.

spores, but also by his subsequent studies and their clear and inspiring presentation.

Kunkelia, gen. nov.

Cycle of development includes subcuticular pycnia and subepidermal telia.

Pycnia conical or columnar, the hymenium applanate; ostiolar filaments wanting.

Telia caeomoid, erumpent, applanate, more or less indefinite in outline, without peridium or paraphyses. Teliospores catenulate, globoid or some elongated, 1-celled; wall colorless or pale, verrucose.

Kunkelia nitens (Schwein.), comb. nov.—*Aecidium nitens* Schwein. Schrift. Nat. Gesell. Leipzig **1**:69. 1822 (type on *Rubus* "*strigosus*," error for *R. Enslenii*, Salem, N.C.); *Caeoma luminatum* Link in Willd. Sp. Pl. **6**[2]:61. 1825 (founded on *A. nitens* Schwein.); *Caeoma* (*Aecidium*) *luminatum* Schwein. Trans. Amer. Phil. Soc. n. ser. **4**:293. 1832 (founded on *A. nitens* Schwein.).

VIII. IDAEI[10] (raspberry)

21. R. OCCIDENTALIS L. (*R. idaeus americanus* Torr.), black raspberry.—NEBRASKA: Peru, May 24, 1900, *John L. Sheldon;* INDIANA: Bourbon (cult.), May 22, 1889, *J. H. Parks;* NORTH CAROLINA: Leicester, June 12, 1909, *B. B. Higgins* (Barth. Fungi Columb. 2937); NEW YORK: Hempstead, Long Island, May 13, 1916, *Percy Wilson* 237; Sparrow Bush, Orange County, May 29, 1916, *Percy Wilson* 255; SOUTH DAKOTA: Lake Oakwood, June 1890, *Miss Stelter.*

XV. URSINI (western dewberry)

71. R. VITIFOLIUS Cham. and Schlecht.—CALIFORNIA: Chico, April 11, 1903, *E. B. Copeland* 3936 (SYDOW, Ured. 1785); Glendora, Los Angeles County, April 10, 1909, *C. F. Baker* 5273; OREGON: La Grand, July 20, 1914, *C. C. Cate* (cult., loganberry).

74. R. MACROPETALUS Dougl.—BRITISH COLUMBIA: Agassiz, June 1913, *James R. Weir* 84.

[10] The numbered divisions of the hosts and the numbers before the species are those employed by RYDBERG in his monograph of *Rubus* in N. Am. Flora **22**:428. 1913.

XVII. Discolores (sand blackberry)

77. R. cuneifolius Pursh.—North Carolina: Raleigh, without date, *F. L. Stevens* 136; Alabama: Auburn, April 26, 1914, *Fred. A. Wolf;* Florida: Lake City, April 13, 1900, *H. H. Hume* 16; Lake City, April 29, 1896, *P. H. Rolfs* 25, same without date 38.

XVIII. Arguti (high blackberry)

78. R. sativus (Bailey) Brainerd.—Indiana: Daleville (cult.), June 8, 1914, *Leslie V. Shoemaker.*

79. R. nigrobaccus Bailey (*R. villosus* Bigel. not Thunb., Ait., or Bailey, collections often labelled *R. allegheniensis*).—Maryland: Rosecroft, Prince George County, May 24, 1910, *E. Bartholomew* (Barth. Fungi Columb. 3238); New York: Arkville, Delaware County, May 30, 1915, *Percy Wilson* 69; Orient, Long Island, June 2, 1915, *Roy Latham* 625; Walden, Orange County, June 20, 1908, *M. E. Cummings;* Ohio: College Hill near Cincinnati, May 15, 1899, *W. H. Aiken* (Sydow, Ured. 1389); Kentucky: Dayton, June 21, 1910, *E. Bartholomew* (Barth. Fungi Columb. 3327); Indiana: Madison, May 6, 1910, *A. G. Johnson;* Wirt, May 7, 1910, *A. G. Johnson;* Illinois: Pine Hills, Union County, April 24, 1882, *A. B. Seymour* (Rab.-Wint. Fungi Eur. 3220*a*); Anna (cult.), June 13, 1888, *F. S. Earle* (Seym. and Earle, Econ. Fungi 27); Missouri: Columbia, May 1886, *Tracy* and *Galloway*, May 25, 1911, *G. M. Reed* 795; Cedar Gap, Ozark Mountains, alt. 1675 ft., May 22–June 3, 1911, *O. E. Lansing, Jr.* 2968; Kansas: Manhattan, May 23, 1889, *Miss May Varney* (Kellerm. and Sw. Kans. Fungi 31); Louisville, June 1912, *E. Bartholomew* (Barth. N. Am. Ured. 605), May 1912, *E. Bartholomew* (Barth. Fungi Columb. 4233); Minnesota: Minneapolis, June 17, 1914, *Bartholomew* and *Holway* (Barth. N. Am. Ured. 1113, Barth. Fungi Columb. 4629); Iowa: Charles City, May 30 and June 20, 1882, *J. C. Arthur;* Decorah, June 11, 1883, *E. W. D. Holway;* Decorah, June 2, 1886, *E. W. D. Holway* (Barth. N. Am. Ured. 211); Decorah, June 3, 1886, *E. W. D. Holway;* Fayette, May 3, 1908, *Guy West Wilson;* Oregon: Freewater (cult., northeastern section of state), June 27, 1913, *F. D. Bailey.*

81. R. ARGUTUS Link (*R. Andrewsianus* Blanch.).—NEW YORK: Hunter Island, New York City, June 14, 1912, *F. D. Fromme* 12, June 18, 1916, *Percy Wilson* 294; White Plains, June 7, 1914, *Percy Wilson;* Bedford Park, New York City, June 9, 1915, *Percy Wilson* 77; Cold Spring Harbor, Long Island, June 13, 1915, *Percy Wilson* 79.

84. R. FRONDOSUS Bigel.—NEW YORK: Sparrow Bush, Orange County, May 31, 1916, *Percy Wilson* 262.

89. R. CANADENSIS L. not A. Gray.—NEW YORK: Arkville, Delaware County, July 6, 1915, *Percy Wilson* 95.

XIX. PROCUMBENTES (dewberry)

99. R. ABORIGINUM Rydb.—TEXAS: Houston, March 6, 1914, *Arthur* and *Fromme* 6108.

102. R. PROCUMBENS Muhl. (*R. canadensis* A. Gray, not L., *R. subuniflorus* Rydb., *R. villosus* Ait.).—NEW YORK: Van Cortlandt Park, New York City, April 25, 1912, *F. D. Fromme* 29; White Plains, June 7, 1914, *Percy Wilson* 2; Williamsbridge, New York City, June 10, 1914, *Percy Wilson* 4; Pleasantville, Westchester County, May 14, 1915, *Percy Wilson* 63; Hunter Island, New York City, May 23, 1915, *Percy Wilson;* Mamaroneck, Westchester County, June 6, 1915, *Percy Wilson* 76; Yonkers, May 27, 1916, *Percy Wilson* 250; Sparrow Bush, Orange County, May 29, 1916, *Percy Wilson* 257; Ithaca, May 30, 1906, *Reddick* and *Frazer;* NEW JERSEY: West Englewood, Bergen County, June 19, 1915, *Percy Wilson* 83; PENNSYLVANIA: Lancaster, May 31, 1910, *E. Bartholomew* (Barth. Fungi Columb. 3239); MARYLAND: High Island, near Washington, May 1894, *P. A. Rydberg;* Cabin John Bridge, June 15, 1910, *Bartholomew* and *Swingle* (Barth. Fungi Columb. 3524); DISTRICT OF COLUMBIA: Takoma Park, May 1898, *C. L. Shear* 1568; MINNESOTA: Nichols, Aitkin County, June 1892, *E. P. Sheldon;* NEW HAMPSHIRE: Temple, June 20, 1888, *A. B.* and *A. C. Seymour* (Seym. and Earle Econ. Fungi 28); CONNECTICUT: Central Village, June 20, 1903, *John L. Sheldon;* NEW JERSEY: Newfield, June 1874, *J. B. Ellis* (Thüm. Myc. Univ. 446); Newfield, June 1893, *J. B. Ellis* (Ellis and Ev. Fungi Columb. 57); DELA-

WARE: Newark, May 15, 1907, *H. S. Jackson* 1620; Newark, June 6, 1907, *Mel. T. Cook* 1661; INDIANA: Greencastle, May 1893, *L. M. Underwood* (Und. Ind. Flora 19); Lafayette, May 21, 1899, *Wm. Stuart;* Brookville, May 8, 1915, *C. A. Ludwig* (Barth. N. Am. Ured. 1411; Barth. Fungi Columb. 4926).

103. R. ENSLENII Tratt.—SOUTH CAROLINA: without locality or date (*Ravenel*, Fungi Car. 1:91); GEORGIA: Darien, without date (*Ravenel*, Fungi Am. 276); NORTH CAROLINA: Salem, without date, *L. D. Schweinitz.*

(?) RUBUS sp. (mostly cultivated blackberry).—MARYLAND: Beltville, May 24, 1916, *H. S. Coe;* MISSOURI: Columbia, May 7, 1905, *H. S. Reed;* OKLAHOMA: Stillwater (cult.), May 14, 1915, *C. D. Learn.*

XX. HISPIDI (running swamp dewberry)

108. R. HISPIDUS L.—NEW YORK: Hempstead, Long Island, May 13, 1916, *Percy Wilson* 234.

XXI. TRIVIALES (southern dewberry)

109. R. LUCIDUS Rydb. (reported in N. Am. Flora 7:181 under *R. trivialis*).—SOUTH CAROLINA: Aiken, March 15, 1909, *Arthur* and *Kern;* FLORIDA: Lake City, March 30, 1895, and February 17, 1906, *P. H. Rolfs;* St. Augustine, March 27, 1903, *E. W. D. Holway* (Barth. N. Am. Ured. 507).

110. R. TRIVIALIS Michx.—FLORIDA: Lake City, February 1896, *P. H. Rolfs* 23; LOUISIANA: New Orleans, February 24, 1913, *E. Bethel.*

111. R. CARPINIFOLIUS Rydb. (reported in N. Am. Flora 7:181 under *R. trivialis*); TEXAS: Austin, February 27, 1901, *W. H. Long* (Barth. Fungi Columb. 1622), March 14, 1901, *W. H. Long* (Barth. N. Am. Ured. 1504), March 16, 1901, *W. H. Long;* Huntsville, without date, *Carl Hartmann*, communicated F. D. HEALD.

DISTRIBUTION: Central Florida to southern Texas northward to Baltimore, Maryland, and central Illinois, still farther north through the prairie region west of the Mississippi River nearly to the Canadian boundary, and along the Atlantic coast within about

100 miles of the sea as far as New York, then nearer to the sea as far as the coast of New Hampshire, also along the Pacific coast within 100 miles of the sea from southern California to southeastern extension of Alaska, the distance from the sea narrowing northward.

Kunkelia Rosae-gymnocarpae (Dietel), comb. nov.—*Caeoma Rosae-gymnocarpae* Dietel, Hedwigia **44**:334. 1905; *Gymnoconia Rosae-gymnocarpae* Arth. N. Am. Flora **7**:181. 1912.

ROSA GYMNOCARPA Nutt.—CALIFORNIA: Santa Cruz, without date, *C. L. Anderson*, communicated W. G. FARLOW; Modoc and Lassen Counties, "killing wild rosebushes," without date, communicated W. G. FARLOW; Jackson, Amador County, without date, *Geo. Hansen* 1012; Ione, Amador County, March 25, 1896, *Geo. Hansen* 2087.

DISTRIBUTION: From central California to northeastern California.

GYMNOCONIA INTERSTITIALIS (Schlecht.) Lagerh. Tromsö Mus. Aarsh. **16**:140. 1894.—*Caeoma* (*Uredo*) *interstitiale* Schlecht. Horae Phys. Berol. 96. 1820 (type on *Rubus arcticus* L., Kamchatka); *Uredo interstitialis* Schlecht. Horae Phys. Berol. 96. 1820 (variant of the preceding name); *Puccinia Peckiana* Howe, Peck, Ann. Rep. N.Y. State Mus. **23**:57. 1872 (type on *Rubus occidentalis* L., New Baltimore, New York); *Puccinia tripustulata* Peck, Ann. Rep. N.Y. State Mus. **24**:91. 1872 (type on *Rubus "villosus," Greig*, New York); *Uredo luminatum* Thüm. Bull. Soc. Imp. Nat. Moscou **55**:85. 1880 (type on *Rubus saxatilis* L., Minussinsk, Siberia); *Caeoma nitens* Burrill, Bull. Ill. Lab. Nat. Hist. **2**:220. 1885 (type on *Rubus occidentalis* L., et al., McLean County, Illinois); *Uredo* (*Caeoma*) *nitens* DeToni in Sacc. Syll. Fung. **7**:866. 1888 (type on *Rubus saxatilis* L., et al., Asiatic Siberia); *Puccinia interstitialis* Tranz. Hedwigia **32**:259. 1893 (founded on *Caeoma interstitiale* Schlecht. and *Puccinia Peckiana* Howe, supported by cultures on *Rubus saxatilis*, Petrograd, Russia); *Dicaeoma tripustulata* Kuntze, Rev. Gen. 3^3:467. 1898 (founded on *Puccinia tripustulata* Peck); *Gymnoconia Peckiana* Trotter, Fl. Ital. Crypt. 1^{12}:338. 1910

(founded on *Puccinia Peckiana* Howe and *Caeoma interstitialis* Schlecht., with *Rubus saxatilis* cited) *Gymnoconia Peckiana* Kleb. Krypt. Fl. Brand. 5*a*:665. 1913.

A. Hosts for aecia

IV. Arctici (northern dwarf raspberry)

4. R. stellatus Smith.—Alaska: Unalaschka (Bernhardi herbarium at Mo. Bot. Garden).

5. R. acaulis Michx. (distributed as *R. arcticus*).—Yukon: White Horse Rapids, June 16, 1899, *J. B. Tarleton*.

VIII. Idaei (raspberry)

21. R. occidentalis L. (*R. idaeus americanus* Torr.), black raspberry.—Vermont: Charlotte, June 12, 1880, *C. G. Pringle* 1128; Burlington, June 11, 1891, *Collins* F1363; Ontario: London, May 20, 1911, *J. Dearness* 1838 *c;* Glenora, June 7, 1912, *J. Dearness* (Barth. N. Am. Ured. 1208); Michigan: Ann Arbor, June 6, 1916, *C. A. Ludwig* 131; Ohio: Olena, Huron County, June 2, 1902, *O. E. Jennings* (Kellerm. Ohio Fungi 67); Massachusetts: Granville, June 1883, *A. B. Seymour;* Connecticut: Central Village, June 28, 1903, *John L. Sheldon;* New York: Onondaga Valley, June 1885, *L. M. Underwood;* Ithaca, June 27, 1907, *Whetzel* and *Barrus;* West Virginia: Seneca, May 30, 1904, *John L. Sheldon* 25; Morgantown (cult.), June 8, 1904, *John L. Sheldon* 502.

32. R. strigosus Michx. (*R. idaeus aculeatissimus* Rob. and Fern.), red raspberry.—Vermont: Burlington, June 10, 1891, *Collins* F1362; Burlington, June 14, 1893, *L. R. Jones;* Burlington, June 10, 1898, *W. A. Orton* F1807; New Brunswick: Salisbury, July 2, 1905, *C. L. Moore* 9; New York: Lyndonville, June 1, 1886, *C. E. Fairman;* Massachusetts: Newton, 1880, *W. G. Farlow* (Ellis, N. Am. Fungi 277, 278, Roumeguère, Fungi Gall. 874).

XVIII. Arguti (high blackberry)

79. R. nigrobaccus Bailey.—Vermont: Without locality, 1893, *A. J. Grout;* New York: Alcove, May and June 1892, *C. L.*

Shear (Shear, N.Y. Fungi 133); Taberg, Oneida County, June 1887, *L. M. Underwood;* Trumansburg (cult.), June 4, 1904, *H. H. Whetzel;* MAINE: Isle au Haut, May 31, 1912, *Arthur* and *Orton* 123; OHIO: Amanda, without date, *W. A. Kellerman* (Rab.-Wint. Fungi Eur. 3225 *b*); Columbus, May 5, 1901, *W. A. Kellerman* 3853 (Kellerm. Ohio Fungi 20); Columbus, June 2, 1901, *W. A. Kellerman* 3854 (Kellerm. Ohio Fungi 19); Johnston, June 18, 1910, *E. Bartholomew* (Barth. Fungi Columb. 3630); MICHIGAN: Portage Lake, Dexter, June 22, 1913, *E. B. Mains* 38.16; WISCONSIN: without locality, 1883, *L. H. Pammel;* Racine, June 26, 1887, *J. J. Davis;* Madison, May 24, 1911, *E. T. Bartholomew* (Barth. N. Am. Ured. 1007, Barth. Fungi Columb. 3911); ILLINOIS: Oregon, June 16, 1885, *M. B. Waite;* Peoria, June 15, 1894, *F. E. McDonald;* INDIANA: Greencastle, May 1878, *Mel. T. Cook;* Lafayette, June 7, 1894, *Miss K. E. Golden;* Greencastle, July 5, 1895, *Guy West Wilson;* Lafayette, May 18, 1896, *Miss Lillian Snyder;* Greencastle, May 27, 1897, *Mel. T. Cook;* Lafayette, April 28, 1898, May 19, 1899, *J. C. Arthur;* Lafayette, May 30, 1909, *Miss Evelyn Allison;* South Bend, June, 1909, *Miss Clara Cunningham;* Lafayette, June 6, 1911, *E. Trager;* Lafayette, June 21, 1912, *C. A. Ludwig;* Indianapolis, June 4, 1912, *N. K. Thompson;* VIRGINIA: Rosalyn, May 28, 1910, *C. L. Shear* (Barth. N. Am. Ured. 106).

81. R. ARGUTUS Link (*R. Andrewsianus* Blanch.).—MASSACHUSETTS: Barre, June 3, 1899, *Harold B. Smith.*

91. R. RANDII (Bailey) Rydb.—NOVA SCOTIA: Pictou, August 1, 1908, *W. P. Fraser* (accompanied with telia).

(?) RUBUS sp. (mostly cultivated blackberry).—ONTARIO: Muskoka, June 24, 1890, *Macoun;* Prince Edward Island: May 7, 1883, *Macoun;* VERMONT: Colchester, June 28, 1894, *L. R. Jones;* MAINE: Orono, June 1898, *P. L. Ricker;* NEW YORK: Onondaga Valley, June 1889 (Und. and Cook, Cent. Ill. Fungi 51); PENNSYLVANIA: Charter Oak, May 1, 1916; *J. C. Arthur;* WEST VIRGINIA: Morgantown, May 7, 1904, *John L. Sheldon* 24; INDIANA: Lafayette (cult.), May, 1901, *H. B. Dorner;* Broad Ripple (cult.), May 25, 1901, *Mrs. L. D. Dickey.*

B. Hosts for telia

V. Saxatiles (dwarf raspberry)

9. R. pubescens Raf. (*R. triflorus* Rich., *R. canadensis* Torr. not L.).—New Hampshire: Albany, August 1908, *W. G. Farlow;* Wisconsin: Oconto County, July 21, 1909, *J. J. Davis.*

VIII. Idaei (raspberry)

21. R. occidentalis L. (*R. idaeus americanus* Torr.), black raspberry.—Vermont: Burlington, August 20, 1890, *L. R. Jones* F1299; New York: Ithaca, September 30, 1912, *B. B. Higgins* (Barth. Fungi Columb. 4020); Ithaca, August 28, 1902, *H. H. Whetzel;* Poughkeepsie, August 1871, *W. R. Gerard* 853; Enfield near Ithaca, September 15, 1902, *J. M. Van Hook;* Glen near Ithaca, August 12, 1904, *H. S. Jackson;* Junius, September 13, 1904, *H. S. Jackson;* Massachusetts: Mt. Tom, August 20, 1883, *A. B. Seymour;* Newton, *W. G. Farlow* (Ellis, N. Am. Fungi 261); Illinois: Urbana, September 7, 1886, *M. B. Waite* 47.

22. R. strigosus Michx., red raspberry.—New York: Junius, September 16, 1904, *H. S. Jackson.*

XVIII. Arguti (high blackberry)

79. R. nigrobaccus Bailey (*R. villosus* Bigel. not Thunb., Ait., or Bailey, collections often labelled *R. allegheniensis*).—Vermont: Jamaica, September 12, 1890, *A. J. Grout* 426; New York: Alcove, August 1892, *C. L. Shear* (Shear, N.Y. Fungi 67); Alcove, August 1893, *C. L. Shear* (Ellis and Ev. Fungi Columb. 346); Ithaca, September 13, 1911, *B. B. Higgins* (Barth. Fungi Columb. 3631); Forest Home near Ithaca (cult.), July 22, 1906, *H. H. Whetzel;* Michigan: Leland, August 23, 1913, *J. C. Arthur;* Illinois: Urbana, July 29, 1884, *T. J. Burrill* (Seym. and Earle, Econ. Fungi 26); without locality, 1887, *T. J. Burrill* (Ellis and Ev. Fungi Columb. 653); Middlegrove, September 17, 1907, *E. Bartholomew* (Barth. Fungi Columb. 2568); Indiana: Lafayette, October 19, 1895, *Wm. Stuart;* Brookville, September 9, 1916, *C. A. Ludwig* 180.

89. R. CANADENSIS L. not A. Gray (*R. Millspaughi* Britton).—VERMONT: Stratton, August 7, 1894, *A. J. Grout* F30 (reported in N. Am. Flora **7**:181 under *R. vermontanus*); NEW YORK: sphagnum swamps at Junius, September 16, 1904, *Jackson* and *Whetzel;* Old Forge, August 25, 1913, *L. O. Kunkel;* Freeville, September 23, 1902, *C. H. Kauffman;* Malloryville, August 19, 1904, *H. S. Jackson;* Seventh Lake, Adirondack Mountains, August 18, 1909, *B. M. Duggar;* MAINE: Isle au Haut, September 10, 1899, *J. C. Arthur;* MICHIGAN: Neebish Isle, August 25, 1899, *E. T.* and *S. A. Harper;* WISCONSIN: Price County, September 17 and 22, 1911, *J. J. Davis;* WEST VIRGINIA: Cheat Bridge, August 18, 1906, *John L. Sheldon* 2433.

91. R. RANDII (Bailey) Rydb.—NOVA SCOTIA: Pictou, August 1, 1908, *W. P. Fraser* (accompanied with aecia).

DISTRIBUTION: From a southern boundary beginning in the vicinity of Boston, Massachusetts, diverging gradually to about 100 miles from the coast as far as central Maryland, then westward to central Illinois on the Mississippi River, and northward into Ontario and Quebec, especially along the Atlantic coast as far as Prince Edward Island, also on the Pacific coast from the vicinity of Mount St. Elias along the Aleutian Islands and the coast of Behring Sea into Asia. In the eastern hemisphere it occurs in northern Siberia and in northern Europe and the high mountains of central Europe.

In the foregoing lists of hosts and localities all the specimens now in the Arthur Herbarium have been entered. This has been done for three principal reasons. It will enable any person having one of these collections to know what disposition has been made of it in the present study, both as to fungus and host; it will serve to show in detail on what data the conclusions of this paper are founded; and, moreover, it is hoped that the long list of localities and hosts will lead to many germination tests. Such tests are very simple. The spores are dusted on the surface of water, and at intervals of 12–48 hours examined under the microscope to see if the germ tube be long and hyphoid, or short and septate with formation of basidiospores. The work will be enhanced in value

if drawings are made or the germinating spores preserved dry between thin sheets of mica, and if a large specimen of the host be pressed for the purpose of specific determination.

The *Rubus* hosts of the lists were carefully examined by P. A. RYDBERG of the New York Botanical Garden, on January 30, 1917, and the names used are in accordance with his judgment. Of course, no person can name species of *Rubus* with much confidence from leaves alone, as in many collections of the rusts, but the list presents the nearest approach to accuracy at present possible. The species have been listed under the divisions of the genus, and with the serial numbers of the species as given by RYDBERG in his monograph of the genus *Rubus* in the *North American Flora*, better to show the relation of the hosts and their possible susceptibility to the rusts. Whenever the specimen is said to come from a cultivated plant, it has been so indicated.

In only one instance have aecia and telia been found on the same plants. Only from Van Cortlandt Park and a few other places in the vicinity of New York City, and from Glen, New Hampshire, have the germination of the spores been satisfactorily observed. In all other cases the aecia of the *Gymnoconia* and the caeomoid telia of the *Kunkelia* have been separated largely upon arbitrary grounds. The geographical factor in connection with known localities for puccinioid telia has been given much weight, while various other collateral items of information have been utilized. In arriving at a conclusion I have had the valuable assistance of Dr. KUNKEL, who kindly went over all the material with me. One reason for making this assortment in detail is the hope of enlisting the interest of any botanist who may have the opportunity of testing the spore germination in moist air from the localities and hosts named, thus aiding in gradually verifying and rectifying the list.

All present evidence goes to show that the long cycle form on *Rubus* is essentially a northern species, while the short cycle form is essentially southern. Fig. 1 shows the present view regarding geographical distribution. The chart is based entirely upon the data given in the preceding lists of hosts, the southern limit of the *Gymnoconia* being in large part that indicated by the collections of

telia. It will be seen that the two forms overlap along an uncertain line running from eastern Massachusetts not far from the Atlantic coast to northern Delaware, then through northern Virginia and West Virginia, south-central Ohio and Indiana to central Illinois, thence northward along the Mississippi River. On the Pacific coast the region within 100 miles or less from the sea is occupied apparently by the short cycle form from Mount St. Elias

FIG. 1.—Distribution in North America of *Gymnoconia interstitialis* (vertical lines) and *Kunkelia nitens* (oblique lines).

to southern California, and by the long cycle form from Mount St. Elias northward and westward into Asia. No collections are known from the arid region of the plains and Rocky Mountains. To what extent the two forms overlap must be left to the future for decision. There seems to be no doubt, however, that the northernmost area of the continent is occupied by the long cycle form, and the southernmost area by the short cycle form, which appears to be

in accord with tendencies recently pointed out by the writer[11] in studying the rusts of tropical America.

Not much can be said regarding susceptibility of hosts. So far as now known, geographical range is more important in determining the susceptibility of the host than the species of *Rubus*. There appears to be some warrant, however, in thinking that the short cycle form is the one "so destructive to our cultivated blackberries and raspberries in this country," as suggested by KUNKEL.[12]

Probably no other species known are so well adapted for the study of the connection between closely related long and short cycle forms, and their possible evolutionary status.

The rose *Caeoma* of northern California is transferred to the genus *Kunkelia* with some confidence in advance of knowledge regarding the spore germination, partly because no puccinioid form has been found associated with it, and partly because of its general similarity to *Kunkelia nitens*.

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[11] ARTHUR, J. C., Rusts of the West Indies. Torreya **17**:26. 1917.

[12] KUNKEL, L. O., Bull. Torr. Bot. Club **43**:569. 1916.

ARBORES FRUTICESQUE CHINENSES NOVI. II

CAMILLO SCHNEIDER

CLEMATIS CHRYSOCOMA Fr., var. **sericea,** n. comb.—*C. montana* var. *sericea* Fr. apud Finet and Gagnepain in Bull. Soc. Bot. France **50**:525. 1903; Contr. Fl. As. Or. **1**:10. 1905.—*C. Spoonerii* R. and W. in Sarg., Pl. Wils. **1**:334. 1913.

Yunnan boreali-occidentalis: in sepibus ad viam principalem inter Yung-peh-ting et Tai-nao-ko, alt. circ. 2600 m., 3 Julii 1914, *C. Schneider* (no. 3929; flores magni albi, frutex scandens).

According to the material before me it seems impossible to separate *C. Spoonerii*, which is said by the authors to be identical with *C. montana* v. *sericea* Fr., as a distinct species from *C. chrysocoma*. I also collected the type on the eastern slopes of the Lichiang range at about 3600 m., August 1914 (no. 3396). This specimen agrees well with FRANCHET'S description in Bull. Soc. Bot. France **33**:362. 1886. With regard to *C. Spoonerii* the authors (l.c.) say: "It appears to us more closely allied to *C. chrysocoma* Franchet, in which, however, the flowers are pink and produced on the shoots of the current season." According to FRANCHET, "dans le *C. chrysocoma* les tiges adultes demeurent extrêmement raccourcies et produisent, seulement sous leurs sommet, le bourgeon floral." In my specimen (no. 3396) the flowers appear from buds on the older wood as they do in *C. montana* or in typical *C. Spoonerii*, while in a specimen of a cultivated plant (Hort. Chenault, 1911) they are produced on this year's shoots. In a specimen collected by G. FORREST that otherwise agrees well with var. *sericea* we can observe both sorts of flowers. After all, I believe that *C. chrysocoma* sensu stricto represents only a "forma depauperata xerophytica," and that var. *sericea* may be regarded as the phylogenetic type of the species. Of *C. chrysocoma* the flowers are rose pink, while the color is white in those of var. *sericea*, but I also collected a form with pinkish flowers which in its manner of growth is more like var. *sericea* than typical *chrysocoma*. To this pinkish flowered form belong the two following specimens: Szechuan australis, inter

Hoh-si et Te-li-pu, alt. circ. 2000 m., 7 Maji 1914 (no. 1128); and Yunnan boreali-occidentalis, ad latera orientalia montium niveorum prope Lichiang, alt. circ. 2800 m., 4 Julii 1914 (no. 1769).

The only real difference between *C. montana* sensu lato and *C. chrysocoma* sensu lato is furnished by the densely pilose achenes which are glabrous in *C. montana*. The yellowish silky pubescence of *C. chrysocoma* is not a reliable character, because I collected a form with almost entirely glabrous leaves but with distinctly pilose young fruits (in dumetis ad latera orientalia montium niveorum prope Lichiang fu, alt. circ. 3300 m., 19 Julii 1914, no. 1928). Unfortunately, I do not know the color of the flowers, having seen only young fruits. The leaves are very much like those of *C. montana* var. *rubens* Wils., and, I presume, this number represents a new variety of *C. chrysocoma*.

CLEMATIS DELAVAYI Fr. var. **calvescens,** n. var.—A typo praecipue recedit foliolis subtus tantum laxe strigoso-sericeis viridescentibus non argenteo-micantibus margine ex parte distincte lobulato-dentatis, floribus ut videtur paullo minoribus.

Yunnan boreali-occidentalis: in declivibus montium in valle fluminis Yang-tze inter Lichiang et Chung-tien, Augusto 1914, *C. Schneider* (no. 2162; typus in Herb. Arb. Arn. et Hb. Schneider; flores albi).

The leaves of typical *C. Delavayi* are distinctly silvery white beneath, while in var. *calvescens* the under surfaces of the leaves are grayish green.

CLEMATIS UROPHYLLA Fr. var. **obtusiuscula,** n. var.—*C. urophylla* R. and W. in Sargent, Pl. Wils. 1:323. 1913, non Franchet.—A typo praecipue recedit foliolis glabrioribus integerrimis vel parce serrato-dentatis sepalis obtusioribus ovato-oblongis stamina paullo non duplo superantibus.

Szechuan orientalis: in monte Omei, alt. 2000 m., 16 Octobris 1903, *E. H. Wilson* (no. 3121; typus in Herb. Arb. Arn.).—Yunnan australis: prope Mengtsze, in silvis versus orientem, alt. 2300 m., *A. Henry* (no. 11, 347; flores albi).

I have seen only one specimen collected by WILSON, November 1900, in western Hupeh, that agrees with FRANCHET'S description of *C. urophylla* in Bull. Mens. Soc. Linn. Paris 1:433. 1884. The two specimens which have been referred by REHDER and WILSON to this species look rather different. The leaves of no. 11347 are entire, while those of no. 3121 are mostly serrate-dentate at the margins between the base and the apex. The sepals of the type are narrow lanceolate, acuminate, and almost twice as long as the stamens.

The flowers of var. *obtusiuscula* are not yet open, but the sepals are distinctly obtuse and not much longer than the stamens and carpels, which are identical with those of the type. Both numbers look very much alike. The inflorescences are the same as in typical *C. urophylla*.

Clematis (Sect. VIORNA Prtl., ser. CONNATAE Koeh.) **Kockiana,** n. sp.—Frutex scandens habitu *C. lasiandrae;* ramuli floriferi striato-sulcati, laxe villosuli. Folia ternata, longiter petiolata; foliola terminalia ovata, basi rotundata vel subcordata, apice subito acuminata, 5–9.5 cm. longa, 2.5–5 cm. lata petiolulis ad 2 cm. longis, superne satis viridia in costa nervisque lateralibus plus-minusve strigulosa, facie subglabra, subtus paullo discoloria, in costa nervisque lateralibus laxe sericeo-pilosa, venis elevatis conspicuis, margine inaequaliter subcrenulato-dentato-serrata, lateralia similia, paullo inaequilateralia, minora, brevius petiolulata. Inflorescentia axillaris, pedunculo quam petiolus breviore ad 3 cm. longo sustenta, paniculata, satis compacta, 3–13-flora et pedunculo incluso ad 10 cm. longa, sericeo-villosula, bracteis variabilibus partim foliaceis partim parvis lanceolatis instructa; pedicelli graciles, ut pedunculus sed densius pilosi, floribus vix vel paullo longiores; flores nutantes; sepala conniventia, apice revoluta, ovato-oblonga, 12–14 mm. longa, 5–6 mm. lata, in vivo flavescentia, sed extus versus basim purpureo-violacea, extus sericeo tomentella, margine tomentosa, intus glabriuscula; stamina exteriora sepalis vix breviora, ad 14 mm. longa, filamentis planis linearibus margine (et etiam in dorso partim) ima basi excepta dense longeque sericeo-pilosis quam antherae glabrae circ. $3\frac{1}{2}$-plo longioribus, interiora breviora, filamentis parte inferiore nudo dilatatis antheris vix ultra duplo longioribus, connectivo in dorso interdum pilis paucis praedito; carpella sericea, in stylum argenteo-plumosum staminibus interioribus subaequilongum desinentia. Achaenia ignota.

Yunnan boreali-occidentalis: in dumetis ad latera orientalia montium niveorum prope Lichiang-fu, alt. circ. 3200 m., 6 Septembris 1914, *C. Schneider* (no. 3898; typus in Herb. Schneider).

This species has the leaves of *C. urophylla* Fr. and the inflorescences and flowers of *C. lasiandra* Maxim., the sepals of which are much more glabrous. The plant is named in compliment to Rev. A. KOCK of the Pentecostal Mission at Lichiang-fu, in appreciation of valued service rendered to the author during the summer of 1914.

Mahonia Alexandri, n. sp.—Arbuscula ad 3 m. alta; ramuli hornotini flavo-viridi, dense foliati. Folia jugo infimo incluso 12-15-juga, ad 32 cm. longa, ad 28 cm. lata, rhachi lateraliter sulcata, jugis inter se 1.5–2 cm. distantibus; foliola lateralia sessilia, anguste lanceolata, versus apicem et basim folii paullo minora, ceterum subaequalia, crasse coriacea, laevia, superne dilute viridia, fere enervia, subtus pallidiora, in sicco flavescentia, 3—nervia, nervis secundariis fere invisibilibus, basi leviter inaequilaterali rotundato-subcordata, apice acuminata, spinosa, utrinque satis crasse sinuato-spinoso-dentata, dentibus distantibus 3–5 divaricatis 1.5–3 mm. longis, basalibus exceptis 4–7 cm. longa, versus basim 1. 2–1. 7 cm., lata, terminalia simillima, breviter petiolulata; stipulae in basi dilatata petioli lineari-lanceolatae. Inflorescentiae densiflorae, fructiferae ad 13 cm. longae; flores ut videtur lutei, ? 8 mm. diametientes; pedicelli 2–4 (–5) mm. longi, bracteis oblongis satis obtusis aequilongis vel paullo brevioribus suffulti; sepala 3 externa minima, late triangularia, 3 media late ovato-oblonga longiora, 3 interna maxima circ. 8 mm. longa, late ovato-elliptica, obtusa; petala late obovata, circ. 6 mm. longa, apice incisa, basi contracta, glandulis 2 subparvis instructa; stamina normalia, connectivo satis obtuso, filamentis edentatis quam antherae fere duplo longioribus; ovarium ovatum, in stylum attenuatum, ovulis 4–5 sessilibus instructum. Fructus globosi, valde pruinosi, seminibus maturis 2–3, stylo brevi instructi.

Szechuan australis: inter oppida Yen-yuan Hsien et Yung-ning inter viculos Wo-lo-ho et Cho-so, ad latera montium, alt. circ. 2600 m., 15 Junii 1914, *C. Schneider* (no. 1588; typus in Herb. Arb. Arn. et Hb. Schneider; arbuscula ad 3-metralis, fruticeta parva formans); Yunnan australis: prope Mengtsze, Lao Kwei-chou (?), 1 Novembris, *A. Henry* (no. 10309; frutex ad 1.8 m. altus; in Herb. Hort. Bot. New York).

My specimens with ripe fruits are identical with the flowering ones of HENRY. It is a distinct species, probably most closely related to *M. caesia* Schn. I take much pleasure in naming this species for my friend Mr. ALEXANDER SCHÖNBAUER of Vienna, in grateful recognition of the important services he has rendered to the dendrological society of Austria and Hungary during my absence from Europe.

Mahonia caesia, n. sp.—Frutex 1–3 m. altus; ramuli (indistincte reticulata costa subtus elevata) juveniles brunnescentes, leviter

pruinosi. Folia jugo infimo incluso 6–8-juga, jugis 1.5–2.5 cm. inter se distantibus, ad. 25 cm. longa (vel in surculis interdum fere duplo majora jugis distantioribus), rhachi tereto plusminusve glaucescenti; foliola lateralia sessilia, anguste lanceolata, versus apicem et basim folii paullo minora, ceterum subaequalia, crasse coriacea, laevia, utrinque in sicco concoloria, flavo-viridia, sed omnino glaucescentia (plusminusve caesia), basi subtruncato-obtusa vel latere inferiore rotundata, paullo inaequilateralia, apice breviter acuminata, spinosa, margine utrinque plusminusve undulato-sinuato-spinoso-dentata, dentibus 5–9 satis crassis subdivaricatis 1.5–2 mm. longis, basalibus valde minoribus ovatis vel rectangularibus exceptis 5–9 cm. longa, 1.2–2 cm. lata, terminalia similia; stipulae jugo infimo approximatae, lineares. Inflorescentiae ignotae.

Yunnan boreali-occidentalis: ad latera montium inter Lichiang-fu et fluminem Yang-tze ad viam principalem versus Yung-peh-ting, 3 Julii 1914, *C. Schneider* (no. 1723; typus in Herb. Arb. Arn. et Hb. Schneider; frutex 1–3 m. altus).

The leaves of this *Mahonia* are so well distinguished from those of any other species that I do not hesitate to describe it as a new species without having seen flowers or fruits. At first sight the leaves resemble those of *M. Alexandri*, but they may easily be distinguished from that species by their different color and nervation, and by the different number of leaflets as well as by their terete rhachis in which the leaflets are inserted in a different manner.

Mahonia philippinensis, n. sp.—Frutex ad 4-metralis. Folia 7-juga, ad 26 cm. longa et 10 cm. lata, jugo infimo multo minore basi petioli valde approximato, rhachi satis tenui lateraliter sulcato, internodiis 2–4 cm. longis; foliola coriacea textura subcrassa, superne ut videtur satis dilute viridia, paullo nitentia, subtus in sicco subflavescentia, nervis primariis utrinque distinctis leviter prominulis, lateralia inferiora superioraque quam media plusminusve minora, basalibus minimis ovatis exceptis lanceolata, basi inaequilaterali cuneata vel obtusata, latere inferiore rotundata (subcordata), apice acutissima, margine dentibus 3–4 mm. longis utrinque 4–6 sinuato-dentato-spinosa, minora 3:1 cm. magna, majora ad 7–7.5 cm. longa et 2–2.5 cm. lata, terminalia lanceolata, basi ovata, ceteris similia, sessilia? (in specimine unico viso foliolis 3 terminalibus basi plusminusve confluentibus), ad 6:1 cm. magna.

Inflorescentiae ad 29 cm. longae, laxiflorae; earum bracteae late triangulares, acuminatae, circ. 1.5 cm. longae; flores lutei?, extus rubescentes?, circ. 10 mm. diametientes; pedicelli graciles, 10–12 mm. longi, bracteis ovato-lanceolatis ad 4 mm. longis subacuminatis suffulti; sepala 3 externa minima, ovata, 3 media majora, late ovata, 3 interna maxima, ad 7 mm. longa, late ovata, apice subrotundata; petala circ. 6 mm. longa, obovata, apice subincisa, basi leviter contracta, glandulis 2 normalibus instructa; stamina petalis breviora, apice obtusa, filamentis edentulatis; ovarium ut videtur in stylum distinctum productum, ovulis 4 sessilibus. Fructus ignoti.

Insulae Philippinenses: Luzon borealis, Benguet, Baguio, 13 Novembris 1914, *R. S. Williams* (no. 1460; typus in Herb. Gray; frutex ad 4-metralis, flores lutei, fructus glauci).

This is a very distinct species, with its loose inflorescences and its long pedicels. I have not seen the fruits mentioned in the note of the collector. The texture of the leaves somewhat resembles that of *M. napaulensis* DC., to which it seems to be most nearly related.

Mahonia nivea, n. sp.—Frutex fide cl. Henry 0.9 m. altus. Folia 5–6-juga, ad 42 cm. longa et 15 cm. lata, rhachi lateraliter subsulcato, internodiis 4–5 cm. longis; foliola lateralia sessilia, late ovata, inferiora superioraque quam media subminora, ceterum inter se subaequalia, basi paullo inaequilaterali truncato-rotundata, apice acuta vel breviter acuminata, tenuiter spinosa, minimis basalibus exceptis 5–8 cm. longa et 2.5–5 cm. lata, utrinque distanter breviter indistincte spinoso-serrata, dentibus 4–7 gracillimis 2 mm. longis porrectis, tenuiter coriacea, superne viridia, laxe tenuiter reticulata, subtus albida, pruinosa, laxius elavato-reticulata, terminalia distincte petiolulata, late ovato-subcordata, ad 8:5 cm. magna, infima ovato-orbicularia distanter spinoso-dentata; stipulae non visae. Flores fructusque ignoti.

Yunnan australis: prope Mengtsze, Pi-che-shen, 21 Novembris, *A. Henry* (no. 9863; typus in Herb. Hort. Bot. New York).

According to the note on the label, HENRY also collected "fl. buds" which, however, are wanting in the specimen before me. Nevertheless, the leaves of this *Mahonia* are so distinct that it undoubtedly represents a good new species. It may easily be distinguished from all the species of the old world by the snowy white under surface of its finely serrate sessile leaflets.

SCHISANDRA GRANDIFLORA Hk. f. and Thoms., Fl. Brit. Ind. **1**:44. 1872.—King in Ann. R. Bot. Gard. Calcutta **3**:219, *pl. 69, fig. A*. 1892; *Kadsura grandiflora* Wall., Tent. Fl. Nepal. 10, *pl. 14*. 1824.—The typical *S. grandiflora* has large white or pinkish white flowers about 1 in. or more in diameter, and the male flowers have 7–9 sepals. The anthers are elliptic or ovoid-elliptic, with lateral or subextrorse cells, the filaments of the lower ones being of about the same length as the anthers. By FINET and GAGNEPAIN and also by REHDER and WILSON, some forms from western and southwestern China have been united with *S. grandiflora* which, in my opinion, form a distinct variety that may be described as follows.

SCHISANDRA GRANDIFLORA, var. **cathayensis,** n. var.—*S. grandiflora* Finet and Gagnep. in Bull. Soc. Bot. France **52**: Mém. IV. 48. 1905, pro parte; non Hk. f. and Th.; Contr. Fl. As. Or. **2**:48. 1907, pro parte; REHDER and WILSON in Sargent, Pl. Wils. **1**:412. 1913; *S. chinensis* Diels in Not. R. Bot. Gard. Edinbgh. **7**:398. 1913, non Baillon.—A typo praecipue recedit floribus minoribus vix ultra 2 cm. diametientibus roseis vel sanguineis antheris partim distinctius extrorsis ovato-ellipticis vel fere ovato-subglobosis apice paullo apiculatis vel interdum leviter emarginatis loculis subrectis vel partim satis curvatis.

Yunnan boreali-occidentalis: in vallibus ad latera orientalia montium Tsang prope Tali-fu, alt. 2800–3200 m., Junio–Augusto 1906, *G. Forrest* (no. 4797); in dumetis montium niveorum prope Lichiang-fu infra glaciem magnam, alt. circ. 3500 m., 14 Sept. 1914, *C. Schneider* (no. 2807; fructus maturi rubri); eodem loco, Octobri 1914, *Schneider* (no. 3303); in silvis umbrosis ad angustias montium inter Sung-queh et Teng-chuan, alt. circ. 3200 m., 29 Sept. 1914, *C. Schneider* (no. 2686); Szechuan australis: in regione Yen Yüan Hsien inter viculos Ka-la-pa et Liu-ku, in dumetis ad ripas, alt. circ. 3300 m., 17 Maji 1914, *C. Schneider* (no. 1276; frutex scandens, flores intense rubri); in silvis supra Hua-li ad flum. Yalung, ad angustias montium boream versus, alt. circ. 3800 m., 28 Maji 1914, *C. Schneider* (no. 3936; typus in Herb. Schneider; frutex scandens, flores rubri).—Vide etiam specimina a cl. Wilson in Hupeh occidentali collecta in Pl. Wils., l.c. indicata.

The color of the flowers of the plants collected by myself is almost blood red, while FORREST says "flowers crimson" and WILSON "flowers flesh pink" or "deep fleshy pink." They are smaller than those of typical *S. grandiflora*, and the shape of the anthers seems rather variable, the cells being often almost entirely extrorse and sometimes distinctly curved. In the male flowers I found mostly 6 sepals, but apparently there are about 9, the outermost being

rather small and very deciduous. Regarding the fruiting aments and the shape, texture, serration, and reticulation of the leaves, it is impossible to detect sufficient differences between this variety and the type. The under surface of the mature leaves is often very glaucescent and without a distinct reticulation, which seems to be much more prominent in the leaves of the type and also of var. *rubriflora*. The last one does not, in my opinion, represent a more distinct form than var. *cathayensis*. I think it best to make *S. rubriflora* another variety of *S. grandiflora*, the main characters of which apparently have been somewhat misunderstood by REHDER and WILSON, and I propose the following combination:

SCHISANDRA GRANDIFLORA, var. **rubriflora,** n. comb.—*S. chinensis*, var. *rubriflora* Franchet in Nouv. Arch. Mus. Paris. **8:** 192 (Pl. David. II. 10). 1886; *S. grandiflora* Fin. and Gagnep. in Bull. Soc. Bot. France **52:** Mém. IV. 48. 1905, pro parte, non Hk. f. and Thom.; Contr. Fl. As. Or. **2:** 48. 1907, pro parte.—A typo differt floribus fusco-rubris (?atro-sanguineis) pedicellis fructuum longioribus 6–8 cm. longis antheris fere ut in var. *cathayensi* et etiam saepe satis distincte extrorsis.

Szechuan occidentalis: in dumetis montis Niu-tou, prope Kuan Hsien versus occidentem, alt. 2000–2600 m., 20 Junii 1908, *E. H. Wilson* (no. 921 b; typus in Herb. Arn. Arb.). Vide etiam specimina altera in Pl. Wils., l.c., enumerata.

The flowers of var. *rubriflora* are as large as those of typical *S. grandiflora*, but "very dark red" according to WILSON's notes. The number of the sepals of the male flowers varies from 5 to 7, and I never saw more than 9 in any form of this species. The shape of the leaves is rather variable, as is also the shape of the anthers. The true *S. chinensis* Baillon is a northern plant, and is readily distinguished by its 5–6 stamens with very short filaments or almost sessile anthers. The male flowers of *S. grandiflora* always possess more than 10 stamens, the filaments of the lower ones becoming almost as long as the anthers.

ARNOLD ARBORETUM
JAMAICA PLAIN, MASS.

CURRENT LITERATURE

NOTES FOR STUDENTS

Temperature and respiration rate.—BLANC[1] has studied the effect of sudden changes in temperature upon the rate of respiration of plant parts. He reviews the work of ZIEGENBEIN[2] with germinated seeds of *Vicia Faba*, and that of PALLADIN[3] with etiolated seedlings of the same species. These two investigators, although working with very similar material, came to very different conclusions as to the influence of sudden changes of temperature upon the rate of respiration. PALLADIN's conclusion that passing from a low temperature or from a high temperature to a medium temperature excites the respiration is considered doubtful on account of the fact that, previous to the change in temperature, the seedlings had been cultivated at different temperatures on sugar solutions.

BLANC worked with the embryos of *Phaseolus vulgaris* deprived of their cotyledons, with the ends of etiolated seedlings of *Vicia Faba*, and with young leaves of *Secale cereale*. The *Vicia* seedlings had previously been cultivated on 10 per cent saccharose or 5 per cent glucose solutions. The *Phaseolus* embryos and *Secale* leaves were used both with and without previous cultivation on 10 per cent saccharose solution. Raising the temperature at which the experiment was conducted invariably increased the rate of respiration, and lowering the temperature always decreased the rate. After having undergone one such change of temperature, samples of the material studied were returned to the original temperature for a short period (15–30 mins.). It was found that the rate of respiration during this second period at a given temperature was higher than that during the first period whenever the temperature had been raised during the intervening period, and lower whenever the temperature had been lowered during the intervening period.

In a third series of experiments, embryos of *Phaseolus vulgaris* and leaves of *Secale cereale* were changed from one temperature to a temperature about 20° C. warmer or 20° C. cooler, and the rate of respiration was determined for 3 successive 20-minute periods at the new temperature in comparison with the

[1] BLANC, M. L., Recherches experimentales sur l'influence des variations de température sur la respiration des plantes. Rev. Gén. Bot. **28**:65–79. 1916.

[2] ZIEGENBEIN, E., Untersuchungen über den Stoffwechsel und die Athmung keimender Kartoffelknollen sowie anderer Pflanzen. Jahrb. Wiss. Bot. **25**:595–596. 1893.

[3] PALLADIN, W., Influence des changements de température sur la respiration des plantes. Rev. Gén. Bot. **11**:241–257. 1899.

rate at the original temperature. It was found that after a change in temperature, the corresponding change in respiratory activity took place only gradually, apparently not having reached an equilibrium even at the end of the third 20-minute period. Although the main point is proved, the value of this part of the work would have been increased by continuing the observations over a longer time.

Many students will regret that the author did not study oxygen consumption as well as the production of CO_2, to see whether the respiratory coefficient was altered by temperature changes. It should be remembered, too, that the conclusions reached may not hold good for other sorts of material, such as dormant seeds, the germination of which is greatly stimulated by alternations of temperature.—G. T. HARRINGTON.

Ecology of bryophytes and lichens.—Ecological studies of liverworts and mosses have not been numerous in the past, largely because bryologists have not been interested in ecology and ecologists have not been sufficiently acquainted with bryophytes. There are also some difficulties peculiar to the application of ecological principles to these plants. Some of these have been pointed out by WATSON[4] in attempting, among other things, to define a xerophytic bryophyte. This he decides must be a plant capable of withstanding long periods of dryness and of having at the end of such periods sufficient living cells to enable it to resume its growth quickly when water becomes available. He proceeds to consider the "xerophytic adaptations" under the two principal heads of structures causing (1) reduction of water output and those resulting in (2) water storage. The former is accomplished by such means as cushion forms, investments of dead cells, thick cell walls, leaf arrangement, and capillary structures; the latter by water sacs, water-storing cells, mucilaginous cells, and succulent tissue. The writer, however, warns us that many bryophytes exhibiting "xerophytic adaptations" are not xerophytes.

A second paper by the same author[5] gives in detail the zonation of bryophytes in a wet heath. The shallow water zone is dominated by *Aneura pinguis*, *Pellia epiphylla*, *Hypnum scorpioides*, and *Sphagnum cymbifolium;* the second zone, just above water level, is dominated by *Aneura multifida;* a third zone consists of *Sphagnum subnitens*, *Hypnum intermedium*, and associated forms, passing imperceptibly into a fourth zone, characterized by *Hypnum cuspidatum*, and closely followed by a fifth zone dominated by *Brachythecium pyrum* and *Cephalozia connivens*. This is frequently the end of the series, although occasionally the drier tussocks show a sixth zone of *Hypnum cupressiforme* var. *ericetorum*. Drainage and the accumulation of humus are the chief

[4] WATSON, W., Xerophytic adaptations of bryophytes in relation to habitat. New Phytol. **13**:149–169, 181–190. 1914.

[5] ———, A Somerset heath and its bryophytic zonation. New Phytol. **14**:80–93. 1915.

factors in determining the succession. The probable history of the heath is well discussed and the diagrams are decidedly good and appropriate.

A remarkable instance of the vitality of moss protonema is recorded by BRISTOL,[6] who found resting protonemal cells, rich in oil, in dry soil stored in air-tight bottles for 46–49 years. In cultures these grew and produced protonema of the ordinary type.

In a series of notes WEST[7] has recorded the bryophytes and lichens found upon trees in parts of Scotland, Wales, and Ireland, and has arranged them according to abundance. He has found the percentage ratio of some of the principal forms to be: *Stereodon cupressiformis* 16, *Parmelia saxatilis* 6, *Isothecium myosuroides* 2, *Frullania dilatata* 2, *Parmelia fuliginosa* 2, *Lecanora tartarea* 2, and *Platysma glaucum* 1.—GEO. D. FULLER.

Variations in wood structure.—Several recent articles have called in question some of the "laws of Sanio" for variation in the size of tracheids in conifers, more particularly that law which states that tracheids increase in size from the pith radially outward until they reach a definite size, which remains constant for the following annual rings. SHEPARD and BAILEY[8] found the gradual increase in size up to 30–60 years, but in succeeding years no constant length was attained. Later the same authors maintained their points in this journal.[9]

Their results were for the greater part confirmed by a detailed study of *Pinus palustris* and *Pseudotsuga* by Miss GERRY,[10] who also finds the longest tracheids in the early spring wood and the shortest in the late wood. LEE and SMITH[11] now supplement this with an extended study of *Pseudotsuga* from British Columbia. Their results, in general, agree with those already cited except that after a gradual and fairly rapid increase up to the age of 50 years the tracheid length varies comparatively little, but tends to increase slightly. They also find an increase in tracheid length up to 42 ft. above the ground, and then a gradual decrease up to 154 ft., where the measurement ceased. It is interesting also to note that trees from the coast region appear to have slightly longer tracheids than those from the mountains.

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[6] BRISTOL, B. MURIEL, On the remarkable retention of vitality of moss protonema. New Phytol. **15**:137–143. 1916.

[7] WEST, W., Ecological notes; chiefly cryptogamic. Jour. Linn. Soc. **43**:57–85. 1915.

[8] SHEPARD, H. B., and BAILEY, I. W., Some observations on the variation in length of conifer fibers. Proc. Soc. Amer. Forest. **9**: 1914.

[9] BOT. GAZ. **60**:66–71. 1915.

[10] GERRY, ELOISE, A comparison of tracheid dimensions in longleaf pine and Douglas fir. Science **43**:360. 1916.

[11] LEE, H. N., and SMITH, E. M., Douglas fir fiber, with special reference to length. Forest Quart. **14**:671–695. 1916.

Extending their work to angiosperms, TUPPER and BAILEY[12] found the average length of their wood elements to be twice that of the corresponding structures in gymnosperms except in the vesselless angiosperms, *Tetracentron*, *Trochodendron*, and *Drimys*, which seem to have the typical gymnospermous length of wood elements. More recently, PRITCHARD and BAILEY[13] examined *Carya ovata* and reached the general conclusion that both in conifers and in woody dicotyledons there is a period in the early stages of the life history during which the woody elements increase in size comparatively rapidly, the length of the period varying in different groups. Furthermore, different types of xylem elements, such as tracheids, wood fibers, and vessel segments, behave very differently, but their size generally fluctuates more or less during the later stages of the development of the stem.—GEO. D. FULLER.

Taxonomic notes.—COOK[14] has made a comparison of the peculiar branching and flowering habits of Cacao (*Theobroma cacao*) and Patachte, formerly referred to *Theobroma*, but recently made the basis of a new genus (*Tribroma*) by COOK.[15] The comparison deals with morphological and ecological features of the two genera, as exhibited under cultivation in eastern Guatemala.

GREENMAN[16] has described a new species of *Senecio* (*S. Hollickii*), collected by BRITTON and HOLLICK in Jamaica in 1908.

GROVE[17] has described, along with other new fungi, a new genus (*Diploöspora*) of Ascomycetes.

ORTON[18] has monographed the North American species of *Allodus*, a genus of Uredinales whose most conspicuous feature is the frequent close association of aecia and telia on the same plant parts, and the absence of distinct uredinia. The most interesting fact in connection with its host relationships is that no host occurs among the Rosales. There are 47 species recognized, including 4 new species and 20 new combinations.

SPRAGUE and HUTCHINSON,[19] in connection with a report upon a collection of African Anonaceae, call attention to the great increase in our knowledge of

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[12] TUPPER, W. W., and BAILEY, I. W., The secondary xylems of gymnosperms and angiosperms. Science **43**:323. 1916.

[13] PRITCHARD, R. P., and BAILEY, I. W., The significance of certain variations in the anatomical structure of wood. Forest Quart. **14**:662–670. 1916.

[14] COOK, O. F., Branching and flowering habits of Cacao and Patachte. Contr. U.S. Nat. Herb. **17**:609–625. *pls. 44–54*. 1916.

[15] Jour. Wash. Acad. Sci. **5**:288. *pls. 46–50, 52, 54*. 1915.

[16] GREENMAN, J. M., A new *Senecio* from Jamaica. Ann. Mo. Bot. Gard. **3**:201, 202. 1916.

[17] GROVE, W. B., New or noteworthy fungi. V. Jour. Botany **54**:217–223. 1916.

[18] ORTON, C. R., North American species of *Allodus*. Mem. N.Y. Bot. Gard. **6**:173–208. 1916.

[19] SPRAGUE, T. A., and HUTCHINSON, J., African Anonaceae. Kew Bull. no. 6. pp. 145–161. *figs. 3*. 1916.

the tropical African flora, as illustrated by this family. In 1868, the date of publication of the first volume of the *Flora of tropical Africa*, only 13 genera and 59 species of Anonaceae were known; while in 1901 there were 23 genera and 170 species recorded, and at present 27 genera are known. In the present paper the limits of certain genera are revised and several species are transferred. New species are also described in *Artabotrys*, *Isolona*, *Oxymitra* (3), *Uvaria*, and *Xylopia*.

WERNHAM[20] has published a new genus (*Pseudomussaenda*) of Rubiaceae from the "Nile-land districts" of tropical Africa. It includes 3 species formerly referred to *Mussaenda*, to which a new species is added.—J. M. C.

Extraction of sap.—GORTNER, LAWRENCE, and HARRIS[21] have repeated and extended the work of DIXON and ATKINS on the extraction of sap from plant tissues. Their primary purpose was to determine something concerning the nature, amount, and regularity of the change in the concentration of the sap extracted from a mass of tissue under continuous pressure. The results secured fully substantiate the conclusions of DIXON and ATKINS that samples of sap pressed from unfrozen tissues cannot be taken as typical of the original concentration of the juices in the tissues. In general, successive samples extracted by continuous pressing become more concentrated. The authors have shown that such, however, is not always the case. In some instances the fluid may become less and less concentrated, for example, extractions from cabbage leaves. In other instances all fractions may be about the same in concentration. The development of the freezing method to render tissues permeable and thereby obtain typical samples of sap has marked a great advance in the study of the properties of vegetable saps.—CHAS. O. APPLEMAN.

A new soil constituent.—An unusual organic soil constituent has been isolated and identified as α-crotonic acid by WALTERS and WISE.[22] This unsaturated acid was found associated with infertility in a Texas soil where drainage is poor, basic compounds deficient, and oxidizing power low. The physical and chemical properties of the purified soil acid agree with the properties of the synthetic acid. The occurrence of this acid in nature had not been certainly established previously. The authors suggest that it may be formed from aliphatic β-hydroxy acids which are produced during the destruction of cellulose, or by hydrolysis of allyl cyanid which occurs in the essential oils of some plants.—CHARLES A. SHULL.

[20] WERNHAM, H. F., *Pseudomussaenda*, a new genus of Rubiaceae. Jour. Botany **54**: 297–301. 1916.

[21] GORTNER, ROSS AIKEN, LAWRENCE, JOHN V., and HARRIS, J. ARTHUR, The extraction of sap from plant tissues by pressure. Biochem. Bull. **5**: 130–141. 1916.

[22] WALTERS, E. H., and WISE, LOUIS E., α-Crotonic acid, a soil constituent. Jour. Agric. Research **6**: 1043–1045. 1916.

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